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# VIRGINIA AGRICULTURAL EXPERIMENT STATION

## The Cedar Rust Disease of Apples Caused by Gymnosporangium Juniperi-Virginianae Schw.

BY

HOWARD S. REED and C. H. CRABILL



VIRGINIA POLYTECHNIC INSTITUTE
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# The Cedar Rust Disease of Apples Caused by Gymnosporangium Juniperi-Virginianae Schw.[1]

By Howard S. Reed and C. H. Crabill.

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[1]Paper No 34 from the Laboratories of Plant Pathology and Bacteriology. Va. Agr. Exp. Sta.

## I.—INTRODUCTION.

Various members of the group of rust fungi have been recognized as the causes of plant diseases for many years. In recent years, attention has been directed to the pathological effects of *Gymnosporangium juniperi-virginianae,* especially in localities where extensive orchards are cultivated in proximity to the red cedar, *Juniperus virginiana.*

Work on the study of this problem has been conducted by the laboratory of Plant Pathology of the Virginia Agricultural Experiment Station since 1910. The financial losses to the apple industry in this State have been very great, and numerous requests have been made for information upon the nature and control of this disease. In the year 1912, the financial loss due to diminished crops was estimated to be upwards of one-half million dollars. The actual loss was much greater than this, however, if the consequent weakening of the tree and lowered vitality of fruit buds be taken into account. It is easy to see these harmful effects in any locality where the disease has been severe for a succession of years. There the trees have made small growth and on account of their weak condition have been attacked by numerous other diseases due to insects and fungi.

In response to the numerous requests for information which was not on hand, plans were made to carry on the work through several seasons in the field and in the laboratory. A field laboratory was accordingly located in the Agricultural High School building at Middletown and equipped for this purpose. It is a pleasure to acknowledge the co-operation and aid which we have received from the people of Middletown in general and the school directors in particular. Much credit is also due the various assistants who have been connected with this work at various times. Messrs. J. S. Cooley, S. F. Coffman, C. H. Chilton, and J. R. Du Shane have rendered aid in one way or another during the progress of the work.

When the work had progressed far enough a report upon the most practical measures of control were issued in the form of a bulletin.[1]

The primary object of this study has been the pathology of the host plant. To that end much attention has been devoted to the physiology of the diseased trees. Studies on the transpiration, photosynthesis, respiration, chemical constitution, and reproductive powers of the tree have been made. Since, however, a proper understanding of the nature and conditions of infection is not evident without a knowledge of the infecting organism, considerable attention has also been directed to the fungus, its spore forms, cycle of development, and growth requirements.

[1]Reed, H. S., Cooley, J. S. and Crabill, C. H. Va. Agr. Exp. Sta., Bul. 203, 1914.

The application of fungicides has not proved to be a practical means of controlling the disease in places where the red cedar trees stand in the neighborhood of orchards. In such cases permanent relief can be obtained by the removal of all red cedar trees in the vicinity.

## II.—BIOLOGICAL RELATIONSHIPS.

The genus Gymnosporangium to which the fungus under discussion belongs is one of the so-called Rusts and is classed in the order Uredinales. It possesses all the ordinary spore forms except the uredospores. Species belonging to this genus are found in North America, Europe, Asia, and northern Africa, but by far the greatest number of known species occur in the first named continent.

The geographical distribution of the cedar rust fungus is naturally within the territory where its two host plants occur. It is found according to Kern[1] from Massachusetts and Ontario west to South Dakota and south to Florida and Texas. Owing to the abundance of the Red Cedar in Virginia and the surrounding states, the fungus reaches a degree of abundance in this region which is hardly to be found elsewhere. So far as known, this species does not occur outside of North America. In the far south of the United States the fungus has been found on *Juniperus barbadensis.* At a nursery in this state this fungus was found upon *Juniperus virginiana var. Scottei, J. virginiana var. glauca, and J. sabina var. fastigiata.*

The species *Gymnosporangium juniperi-virginianae* was first described by Schweinitz in the year 1822. Link described the same thing as *G. macropus* in 1825, and this name was in common use until recently. According to the rule of priority the name given by Schweintz must be regarded as valid.

All species of Gymnosporangium, except one, in which all four forms of spores are unknown, require two host plants for their complete development, i. e., are heteroecious. The species under study requires the Red Cedar, *Juniperus virginiana* for one cycle of development and a pomaceous plant for the other. (Fig. 1.) The pomaceous plants thus far listed as hosts are the cultivated apple *(Pyrus malus)*, the wild crab-apples *(Pyrus angustifolia, Pyrus coronaria, Pyrus ioensis and Pyrus baccata).* The Red Cedar serves as a host during the development of the teleutospores and sporidia; the pomaceous tree, during the development of spermatia and aecidiospores. The stage of the fungus on the Red Cedar was known for many years before proof was afforded that it was one stage in the development of the Roestelia, or " Cluster cup," on the leaves of the pomaceous

[1]Kern, F. D. Bulletin, N. Y. Botanical Garden. 7:474. 1911.

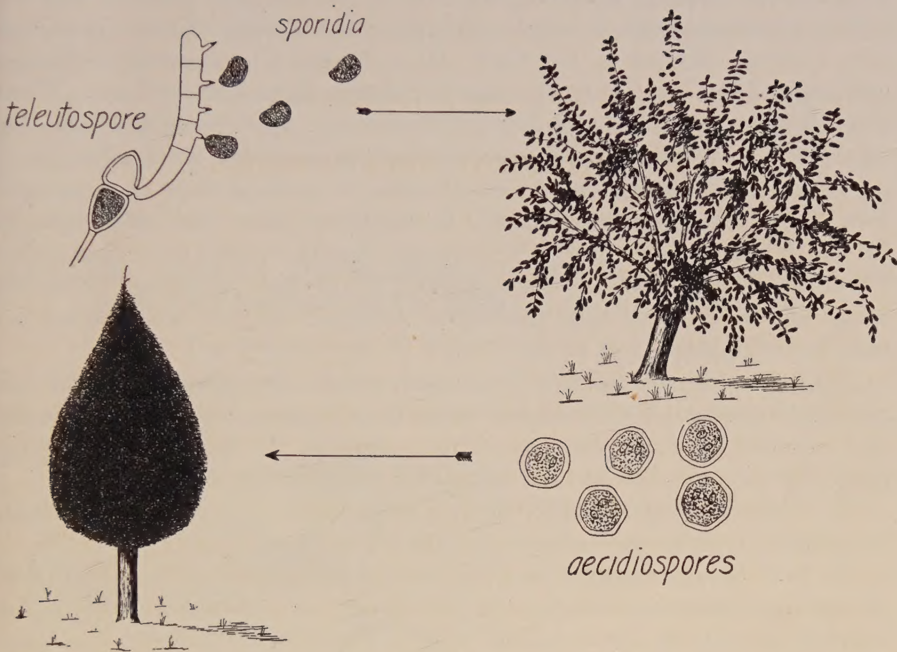


Fig. 1.—The relation of red cedars and apple trees to the cedar rust fungus. The teleutospores produced on the cedars furnish sporidia in the spring which infect the apple foliage. Aecidiospores produced during the summer on the apple foliage reinfect the cedars.

trees. Farlow obtained incomplete proof of the identity of the two fungi in 1877.[1] The identity of the two was fully proved by Thaxter,[2] in 1886. Prior to this time the stage of the fungus found on pomaceous trees was known as *Roestelia pyrata (Aecidium pyratum* of Schweinitz*)*, but Thaxter proved that this stage could only be produced by sowing the sporidia of the *Gymnosporangium* from the cedar tree upon the foliage of the pome. He sowed sporidia March 1st on the leaves of apple trees which had been forced indoors. On March 10 spermogonia were found, and on May 1 aecidia appeared which were those of the fungus formerly called *Roestelia pyrata.*

About the same time Halsted reported a similar experiment showing the identity of the two fungi.[3] In the spring of the year he placed sporidia on leaves of the Wild Crab-apple and of Ralls *(Rawles Janet)*, and Tallman *(Tallman Sweeting)* apples. Spermogonia appeared on the Wild Crab-apple leaves in two or three weeks, and aecidia came somewhat later. He failed, however, to get infection on the foliage of the apple tree, the reason for which is not apparent.

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[1]Farlow, W. G. Anniv. Mem. Boston Soc. Nat. Hist. 1880. p. 35.
[2]Thaxter, R. Proc. Amer. Acad. 22:259. 1887.
[3]Halsted, B. D. Bulletin Iowa Agr. Coll. Dept. of Bot. 1886. p. 59.

Seymour's paper[1] reviewed the work of Farlow and Thaxter and described numerous cases in which orchards in the vicinity of cedar trees had been seriously injured by the Rust. He published a letter from an Illinois orchardist describing the injury observed there. Rome *(Rome Beauty)*, Red June, Hightop Sweet *(Sweet June)*, "Strawberry" and Siberian Crab were mentioned as varieties which were susceptible to cedar Rust infection.

Stewart and Carver[2] made inoculations on apple foliage in Iowa and New York. The inoculations made in the latter place were more successful which was probably due to the fact that they there used more susceptible varieties of apples for experimentation. When they inoculated Maiden's Blush and Wealthy in both states they obtained infection and mature aecidia in both places.

Professor Pammel of the Iowa Agricultural Experiment Station has recorded considerable data on the Cedar Rust fungus in that State. In his first reports[3] he stated that the fungus attacked the foliage of the Wild Crab *(Pyrus coronaria)* but not that of the cultivated apple.

In a subsequent publication[4] he reports that the disease was found upon the foliage of cultivated apples (Wealthy).

In 1907 Heald reported the first results of a study of the fungus *G. juniperi-virginianae* undertaken in Nebraska.[5] He started work with the supposition that the aecidiospores from the apple tree produced the cedar apples which matured in the autumn of the same season, but found that this assumption could not be confirmed, because cedar apples of appreciable size appeared prior to the opening of the aecidia. Two explanations suggested themselves to the author:

1. The fungus is either perennial in the cedar, or
2. The aecidiospores of one season produce the cedar apples which appear in June of the next year, and reach maturity in autumn.

In a later publication[6] he reported that the second of the above assumptions was correct. The infection of the cedars takes place during July, August, and September, but no visible signs of infection can be noted. The mycelium apparently remains dormant during the remainder of the season and no cedar apple is produced until the resumption of growth by the cedar the following spring. The cedar apples become visible in the month of June, grow throughout the summer and are practically mature in size and form at the end of the growing season.

This fungus would therefore appear to be a biennial parasite.

Arthur[7] reports that spores from a peculiar form of *G. juniperi-virginianae* collected at Mammoth Cave, Ky., produced aecidia on *Pyrus malus* and

[1]Seymour, A. B. Trans. Amer. Hort. Society. p. 152. 1886.
[2]Stewart, F. C., and Carver, G. W. N. Y. Agr. Exp. Sta. Rep't. 1895-96. p. 535.
[3]Pammel, L H. Iowa Agr. Exp. Sta., Bul. 13. 1891. Trans. Iowa Hort. Soc. 28:470. 1893.
[4]Pammel, L. H. Iowa Agr. Exp. Sta., Bul. 84. 1905.
[5]Heald, F. D. Science N. S. 26:219. 1907.
[6]Heald, F. D. Neb. Agr. Exp. Sta., 22nd Rep't., p. 105. 1909.
[7]Arthur, J. C. Mycologia, 1:225. 1909.

*P. coronaria*, but not on *Crataegus punctata*. However, spores from a form of the fungus producing small galls upon the red cedar produced aecidia upon the Crataegus.

The present writers have observed that in rare cases a cedar apple may live through three years. This is observed in the case of very small galls posessing only two or three sori. After discharging their spores, such galls may begin to grow again and produce more sori which mature the following spring. This has never been observed in the case of the larger galls.

## III.—THE VERNAL DEVELOPMENT OF THE CEDAR APPLES AND THE SPOROPHYTIC STAGES OF THE FUNGUS.

### 1. The Production of Teleutospores and Sporidia.

The galls on the cedar trees resulting from infection with Gymnosporangium develop and produce spores according to Heald[1] in about 21 months. At the approach of the second winter of their development they are nearly full-sized but usually show no indications of the pits which later develop the sori. During the second winter, however, they appear to undergo considerable development, probably during the mild weather. Those on the exterior of the cedar tree in positions receiving the warmth of the sun develop notably faster than those in the interior of the tree.

During the open weather of late winter and early spring the surface of the cedar galls becomes notably pitted and their original grey-green color begins to be mixed with red and brown. By March 1st in this latitude the sori contain fully formed teleutospores. (Fig. 2.) The extrusion of tendrils of teleutospores is then largely a question of weather conditions. In 1911

Fig. 2.—Mature cedar apples photographed April 2. The teleutosori have just ruptured the cortex preparatory to the projection of tenacles during the next rain.

[1]Heald, F. D. Neb. Agr. Exp. Sta., Rep't. 22:105. 1909.

the first sori began to break on March 15th, in 1913 the bursting began on March 4th. The sori began to break and to extrude tendrils of spores first on the exterior of the tree. Those protected by thick foliage did not develop so early.

Although the sori often burst in March there is usually no extrusion of tendrils until after a warm rain, a condition which is not commonly afforded until April. (Fig. 3.)

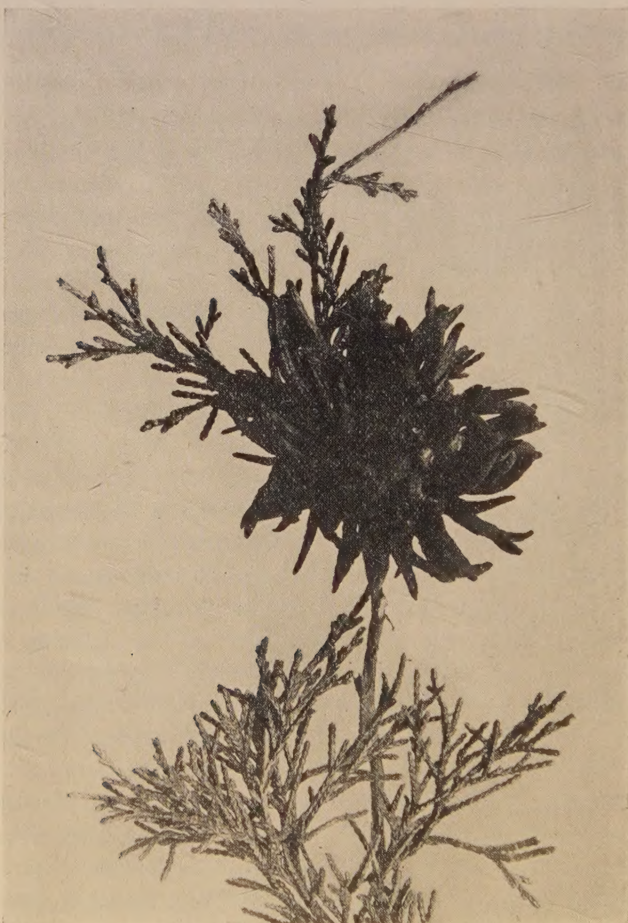

Fig. 3.—A cedar apple with gelatinous teleutospore tentacles extruded. Photographed six hours after a rain on April 20.

The process invariably begins after a rain of at least six hours' duration. Our observations on this point are given herewith.

TABLE I.—*Date at which Tendrils of Teleutospores Began to Appear.*

| Date | Temperature | Highest previous temperature had been |
|---|---|---|
| 1911—April 8th | 5.6° C. | 10.0° C. |
| 1912—April 13th | 13.8° C. | 8.9° C. |
| 1913—April 11th | 13.3° C. | 10.5° C. |

Early in April teleutospores were abundant, but ungerminated, although experiments made April 8, 1911, showed that they would germinate in 18 to 36 hours if put into hanging drop cultures in a warm room. The field records show that teleutospores do not germinate on the cedar tree unless the temperature of the air is somewhat above 10°C, when the tendrils are highly gelatinous and teleutospores are abundant. Examples are given in table II.

TABLE II.—*Date at which Sporidia Were First Found upon the Cedar Trees.*

| Date | Weather | Temperature | Average temperature of preceding 7 days |
|---|---|---|---|
| 1911—April 19th | Rain | 12.7° C. | 6.7° C. |
| 1912—April 16th | Rain | 15.0° C. | 9.5° C. |
| 1913—April 12th | Rain | 11.1° C. | 7.8° C. |

These data show that the germination of the teleutospores is largely dependent upon temperature. The teleutospores may be said to begin germination after the first rain which is warm enough to make the gelatinisation of the sori and consequent extrusion of tendrils conspicuous from some distance.

The color of the sori before they become gelatinous is reddish brown. The tendrils of teleutospores which they extrude are orange color, but the sporidia withdraw the orange colored oil from the teleutospores and the whole tendril becomes much paler in consequence.

The gelatinous tendrils rapidly lose water after rain ceases to fall, the rate depending upon the drying power of the atmosphere.

The next time that rain falls for a period of six or more hours the dried tendril may imbibe water and again become gelatinous. This process is not repeated indefinitely however. The same material does not become gelatinous more than two or three times. After the teleutospores have germinated, their stalks become unified into a hard, non-absorbent mass and remain so as long as the tendril remains attached to the sorus. When subse-

quent rains come, the sorus pushes out more material so that the tendril is elongated by an addition of substance at the base.

This periodicity of spore formation is a point of great importance in studying the scientific and economic problems connected with the fungus, because it makes the time of infection of the apple foliage dependent largely upon weather conditions. Our observations on this periodicity over a period of four years are as follows:

TABLE III.—*Dates on which Cedar Galls Became Gelatinous in the Years 1911 to 1914 Inclusive.*

| | 1911 | 1912 | 1913 | 1914 |
|---|---|---|---|---|
| First gelatinization of sori... | April 19 | April 16 | April 12 | April 28 |
| Second " " " ... | | April 30 | May 16-19 | May 4 |
| Third " " " ... | | May 8 | May 24-28 | May 8 |
| Fourth " " " ... | | May 13 | | |
| Fifth " " " ... | | May 20 | | |
| Sixth " " " ... | | June 6[1] | | |

The rate at which the tendrils are extruded probably depends largely upon the number of rains and the duration of each. The above data show that the contents of the sori are more quickly exhausted in some seasons than in others. On June 30th, 1912, a quantity of teleutospores taken from dried cedar apples was tested but would not germinate. In 1912 the last sporidia were produced after a shower on June 6th, while in 1913 no sporidia were produced after May 28th.

As late as June 13th, 1913, a few mature teleutospores could be found in the cedar galls, yet very few of those found produced sporidia when placed under suitable conditions. On July 3d the cedar galls were black and almost entirely free from tendrils, no rain having fallen since June 28th. Pieces of the hard black tentacles, when teased up, showed a large number of teleutospores, but most of them appeared to be partly collapsed and dead. Tests made in hanging drop cultures showed that these teleutospores were incapable of germination.

Observation shows that sporidia production for the year generally ceases in most places in Virginia early in June.

### 2. The Dissemination of Sporidia.

When the work was started it was thought that it would be possible to make some definite records of the rate at which sporidia are liberated from cedar galls and also of the distances to which they may be carried by air

[1]At this time the formation of tendrils was confined to galls in the tops of the cedar trees; those on the lower branches, being exhausted, produced none.

currents. We found, however, that the results which could be obtained were only roughly quantitative and not truly qualitative.

The traps employed were of three kinds: 1st, the ordinary microscope object slides 25 x 75 mm., covered with a coating of vaseline; these could be taken to the laboratory and examined directly under the microscope; 2nd, large dinner plates filled with distilled water, from which the sporidia could be obtained by centrifuging; 3rd, pieces of clean, absorbent cotton, from which the sporidia were washed with distilled water and centrifuged as above. Each sort of trap was set on poles elevating them some two meters high, where they would approximate the conditions of leaves on the lower branches of apple trees.

The coated slides were, after many trials, abandoned because they were so easily spoiled by rain or dew, the absorbent cotton traps were also abandoned. The plates of distilled water, were, for purely qualitative results, more suitable than the others.

The results, presented in the following table, show the approximate amount of dissemination of sporidia after a rain on April 30th, 1912, which caused the cedar galls to become very gelatinous and produced a large crop of sporidia. The data were obtained by counting the numbers of sporidia on vaseline coated slides as seen with the 4 mm. objective of the microscope after the slides had been exposed one day. Each figure is the result of several counts.

TABLE IV.—*The Time Factor in Relation to the Numbers of Sporidia Caught on Traps.*

| Distance from cedar trees | No. of Sporidia present in field of microscope | | |
|---|---|---|---|
| | After 1 day | After 2 days | After 3 days |
| 500 meters | 7 to 10 | 0 to 7 | 0 |
| 100 meters | 6 to 8 | 7 to 10 | 0 to 3 |
| 5 meters | 5 to 10 | 0 to 7 | 5 to 7 |

The differences, while not great, are of sufficient magnitude to show that there was a diminution of dissemination of sporidia on the three fair days following the rain designated.

The methods as employed were not accurate enough to show differences in the rate of dissemination between windy and calm weather. The sporidia of Gymnosporangium appear to be so universally present in the air of Frederick County (Virginia) that traps exposed at any place during the period of production caught some sporidia.

The conclusion was reached that better information concerning rate of dissemination, distance carried, etc., could be obtained from a study of the amount and time of infection of apple leaves by the disease.

The following data were obtained in an orchard which adjoins a large forest principally of cedars. The orchard lies on the east side of the forest and the prevailing winds are from the southwest. The land is almost level so that no natural barriers deflect the air currents. The results presented in table V were obtained in 1914, which was remarkable for its long drouth. The number of infections on all the leaves on 20 twigs at different distances from the cedars were counted. The notation used in the table ("Leaf No. 1." etc.) means that the leaves were numbered in serial order from the base of that season's growth to the apex. Cluster leaves were excluded. From May 6 until August 1 the rainfall was almost none. Only slight showers occurred on June 27 and July 2. Such a season is unfavorable to cedar rust infection and the injury done was much smaller than in years of normal rainfall. The records obtained are particularly satisfactory because they probably represent the results of a single dissemination.

TABLE V.—*Average Number of Rust Spots Per Leaf on 20 Twigs. July 23, 1914.*

| Distance from cedars | Leaf Numbers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| [1]50 meters | 7.8 | 53.0 | 178.0 | 75.0 | 48.0 | 21.0 | 6.7 | 3.6 | 0.5 | 0.5 | 0.0 |
| 100 meters | 22.0 | 98.0 | 72.0 | 34.0 | 14.0 | 76.0 | 36.0 | 15.0 | 45.0 | 0.0 | 0.0 |
| 200 meters | 15.0 | 31.0 | 22.0 | 14.0 | 3.4 | 1.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| 300 meters | 11.0 | 18.0 | 12.0 | 4.1 | 1.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

The average number of infections per twig is 35.8 at 50 m.; 34.7 at 100 m.; 7.9 at 200 m.; and 4.2 at 300 m.

The above table shows that rust infection decreased rapidly as the distance from cedars increased. At a distance of 800 meters, approximately half a mile, rust infection would be slight.

The seasonal distribution of the rust is also clearly shown in this table. The worst infections took place on the 2nd, 3rd and 4th leaves to unfold, which were young and exposed about May 9, just after a gelatinization of cedar apples on May 6 and 7. Infections subsequent to this were small following very slight showers.

[1]Many of the worst infected leaves had already fallen from these trees. The figures are therefore too low.

### 3. The Susceptibility of Cedar Trees.

The red cedar is usually dioecious. Many farmers recognizing that fact have evolved the idea that only pistillate cedars bore cedar apples and some have even shown some supporting evidence. In the first such case recorded, two cedars of opposite sex stood side by side. The pistillate tree was full of cedar apples, the staminate one was free.

In studying the possible effect of sex on production of cedar apples, 293 trees were carefully examined. All had about equal chances for infection. 39 female trees bore many cedar apples, 31 bore few, and 7 bore none. 29 male trees bore many cedar apples, 12 bore few, and 2 bore none. Many trees bore neither pistillate nor staminate bloom and the sex was therefore not determined. Of these, 86 bore many cedar apples, 74 bore few, and 11 bore none. Of two monoecious trees, one bore many cedar apples, the other few.

These notes show conclusively that sex does not influence infection. In numerous instances of two males standing side by side, one would be full of cedar apples while the other had only a few. The same was true of females, of those of undetermined sex and of those of the juvenile form. The great difference in susceptibility of cedars seems to be individual. Susceptibility also seems to be cumulative. A tree once infected becomes increasingly susceptible from year to year as the increasing numbers of cedar apples indicates. Weakening of the tree by the parasite no doubt accounts for this.

In a paper read at the 1914 meeting of the American Phytopathological Society, Giddings reported that heavy infection of a cedar tree may produce a weakened condition which renders that tree practically immune for one or two seasons[1].

### 4. The Toxic Action of Spray Materials upon Sporidia.

The method used in our studies was that described by Wallace.[2] Microscope object slides were placed in a tray and sprayed with the solutions to be tested. The slides were then drained and allowed to dry until the following day. The tests were made by placing a drop of water containing viable sporidia upon the sprayed slides simultaneously with similar preparations on unsprayed slides. Tests were made both with teleutospores and sporidia, but chiefly with the latter since they are the agents directly concerned in infection.

It was usual to take the sporidia from the tendrils upon which they had formed when the tendrils were passing from the gelatinous to the dried con-

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[1]Giddings, N. J., and Berg A. Phytopathology. 4:401. 1914.
[2]For description of methods used by other investigators, see:
Burrill, T. J. Ill. Agr. Exp. Sta. Bul. 118: 553-608. 1907.
Reddick, D., and Wallace, E. Science 31: 798. 1910.
Wallace, E.; Blodgett, F. M., and Hesler, L. R. Cornell Agr. Exp. Sta., Bul. 290. 1911.

dition, to mix them quickly in a dish of distilled water and to transfer drops to each of the slides to be used.

The slides bearing these drops were placed in a saturated atmosphere in a moist chamber and held for one or two days, the time depending somewhat upon the temperature.

The results of some tests with various spray mixtures upon the teleutospores are shown in table VI.

TABLE VI.—*The Toxic Action of Some Spray Materials Upon the Viability of Teleutospores.*

| Spray material | Date of test | Percent of germination after 3 days |
|---|---|---|
| Bordeaux mixture | April 11, 1912 | 0 |
| Lime sulphur | April 11, 1912 | 50-75 |
| Copper lime sulphur | April 11, 1912 | 0 |
| Iron Bordeaux | April 11, 1912 | 0 |
| Controls (average of three) | April 11, 1912 | 65 |

With the exception of lime sulphur solution the materials tested were able to inhibit germination of the teleutospores. No conclusions are drawn however, because the number of tests was so small.

More extended studies were made upon the toxicity of spray materials for sporidia, and these are presented in table VII.

TABLE VII.—*Effect of Spray Materials on Germination of Sporidia.*

| Kind of Spray | Date of test | Percent of germination |
|---|---|---|
| Bordeaux | April 26, 1911 | trace |
| | May, 9, 1911 | 4 |
| | April 11, 1912 | 0 |
| | April 16, 1912 | 2 |
| | April 23, 1912 | 2 |
| | May 23, 1913 | 0 |
| | May 28, 1913 | 0 |
| | May 30, 1913 | 0 |
| Iron Bordeaux | April 26, 1911 | trace |
| | May 9, 1911 | 6 |
| | April 11, 1912 | 0 |
| | April 16, 1912 | 10 |
| | April 23, 1912 | 7 |
| Pyrox | May 23, 1913 | 0 |
| | May 28, 1913 | 0 |
| | May 30, 1913 | 0 |
| Lime sulphur | April 26, 1911 | 2 |
| | April 23, 1912 | 5 |
| | May 23, 1913 | 0 |
| | May 28, 1913 | 0 |
| | May 30, 1913 | 0 |
| Copper lime Sulphur | April 26, 1911 | 1 |
| | May 9, 1911 | 5 |
| | April 11, 1912 | 0 |
| | April 16, 1912 | 5 |
| | April 23, 1912 | 3 |
| Atomic sulphur | April 26, 1911 | 4 |
| | May 9, 1912 | 3 |
| | April 23, 1912 | 3 |
| Sulfocide | May 23, 1913 | 0 |
| | May 28, 1913 | 0 |
| | May 30, 1913 | 0 |
| Controls | April 26, 1911 | 13 |
| | May 9, 1911 | 48 |
| | April 11, 1912 | 65 |
| | April 16, 1912 | 80 |
| | April 23, 1912 | 33 |
| | May 23, 1913 | 100 |
| | May 28, 1913 | 96 |
| | May 30, 1913 | 25 |

The comparative success of the method is quite evident from an inspection of this table. In general it may be said that each of the toxic agents used was successful in inhibiting practically all germination of sporidia on the slides.

If we compare the percentage of germination of any particular lot of sporidia with the control slides made on the same day, the results appear quite satisfactory, although there was considerable fluctuation in the germination of the control, and the average germination of the controls is somewhat low owing to the low figures obtained in 1911.

The results obtained on slides sprayed with Bordeaux mixture, Pyrox, Lime-sulphur, Copper lime sulphur and Sulfocide are all indicative of strong toxin action. In the trials made in 1913 germination was absolutely inhibited by Bordeaux mixture, Pyrox, Lime sulphur and Sulfocide, while the average percent of germination in that year on the control slides was 74. Atomic sulphur permitted slightly more germination than the other materials used and thus these experiments seem to corroborate those made in the orchard.

It is of course impossible to make too close comparisonsbetween a sprayed slide and a sprayed leaf, because on the latter there may be other factors at work due to the activities of the living leaf. However, these results along with those of the investigators previously mentioned show that this method is quite applicable for the study of these problems.

## 5. The Comparative Anatomy of the Normal Cedar Leaf and the Cedar Apple.

The cedar apple is merely an hypertrophy of a cedar leaf infested by the fungus *G. juniperi-virginianae.* Structurally their tissues are quite similar, those of the cedar apple being mere modified continuations of those of the normal leaf.

According to the U. S. Dispensatory (18th edition, p. 1699) cedar galls are popularly used as anthelmintic in doses of 0.65 to 1.3 grams three times per day. The treatment is not official and is not given in the U. S. Pharmacopoeia.

a. The Cedar Leaf.—As shown in Fig. 4 the cedar needle is for the greater part of its length tightly attached to the twig. Only the apical portion is free.

"The epidermis consists of a layer of flattened elongated cells, with rather thick walls, communicating by simple pits, and on the inner side of the free part of the leaves the epidermis is covered by numerous stomata. On the dorsal side of the leaf the epidermis is underlaid by a simple layer of strengthening sclerenchymatous cells, which are doubled in the corners and also on both sides of the glands, over which the hypodermal layer does not extend. On each side of the fibrovascular bundle is placed a group of tracheid-like, elongated cells with lignified walls and bordered pits as is also seen on each side above and below the vascular bundle of the common leaf axis. These tracheid-like cells show the more or less peculiar curved projections from their walls, a feature characteristic of the dorso-ventral-leaved section of Juniperus. The fibrovascular bundle passes along the ventral side of the gland and contains on the border scalariform cells. The paren-

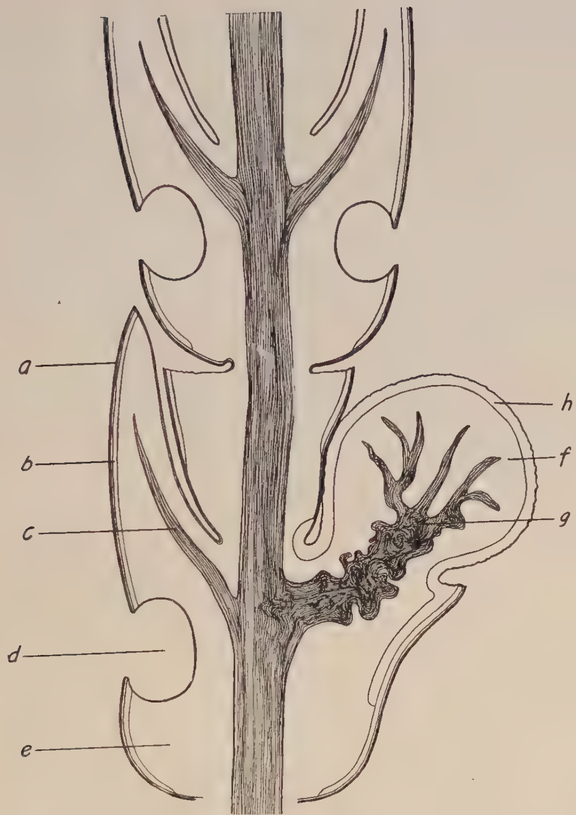


Fig. 4.—Diagram of a longitudinal section of a cedar twig bearing a minute cedar apple collected in June. (a) epidermis of cedar leaf, (b) sclerenchymatous layer, (c) fibrovascular bundle, (d) resin gland, (e) parenchyma, (f) parenchyma of cedar apple, (g) fibro-vascular system of cedar apple, (h) cortex. Note that by an alteration of the parenchyma cells of the leaf this cortical layer has extended down for a considerable distance just beneath the sclerenchymatous layer. The apical portion of the leaf above the infection has died and fallen away.

chymatous tissue is loose, composed of large cells, the palisades normal to the surface."[1]

It will be readily seen that several factors prevent the infection of the cedar needle on its ventral surface. (1) the surface is smooth and offers no lodgment for the aecidiospores, (2) a protecting sclerenchymatous layer underlies the epidermis, (3) stomata are absent. Other factors multiply the possibilities of infection on the dorsal side of the leaf. (1) lodgment for spores is afforded in the axil of the leaf, (2) only a thin epidermis must be punctured by the invading germ tube, (3) stomata exist in this region, (4)

[1]Quoted from Mohr C. (after Filibert Roth). Notes on the Red Cedar. Division of Forestry, U. S. D. A. Bul. 31. 1901.

the axil of the leaf holds water, thus supplying moisture to the germinating spore. All that is necessary then is a degree of warmth sufficient for germination.

b. The Histology of the Cedar Apple.—As we have already noticed, cedar apples do not become mature until about eighteen months after the production of the aecidiospore which causes the infection of the cedar leaf. Whether the aecidiospores infect the cedars as soon as they are produced in the autumn, or whether they rest over winter and infect the cedar the following spring has not yet been conclusively demonstrated. At any rate, the cedar apples resulting from infection by aecidiospores produced in autumn do not become mature and produce teleutospores until one year from the following March or April.

The observations of previous investigators that infection always takes place on the dorsal surface of the cedar leaf, have been confirmed by the writers. The production of a cedar apple is the immediate result. The parenchyma of the leaf is stimulated to excessive growth, the fibrovascular system, which is close to the point of infection, becomes much contorted and branched and radiates through the forming cedar apple. (Fig. 4). For the minute histology of the cedar apple, see the following section.

Cedar apples in all stages of development have been collected, killed, sectioned, and stained with numerous combinations of all the usual stains. Sanford[1] has given us a description of the tissues of the cedar apple. His observations have been substantiated by the work here reported and additional observations have been made. A later study by Wörnle[2] verifies many of Sanford's observations, although he wrongly regards the cedar gall as a hypertrophied twig.

The cedar apple may properly be divided into four portions, (1) the cortex or rind, (2) the parenchyma, (3) the fibro-vascular system, and (4) the fungus.

1. *The Cortex* is a layer 4 to 6 cells thick which covers the cedar apple. Prior to the rupture of the teleutosori the cortex is thickest over these fruiting bodies. The cortical cells are empty, irregularly compressed, and exhibit all the characteristics of dead cells.

2. *The Parenchyma,* which is a modification of the cedar leaf parenchyma, makes up the greater portion of the cedar apple. It consists of a mass of cells which are continuous with and similar to the normal parenchyma cells of the cedar leaf, except that as a result of the stimulation of the fungus they have attained a relatively enormous size. They measure up to 90x150 $\mu$., while the normal parenchyma cells of the cedar leaf measure on a maximum about 30x80 $\mu$. They are thin walled, are for the most part

[1]Sanford, E. Annals of Botany. 1:263. 1888.
[2]Wörnle, P. Forst.—Naturw. Zeit. 3:68, 129. 1894.

oblong oval in shape and lie with their long axes coincident with the radii of the cedar apple. They are as closely packed together as their oval shape allows, but, owing to their large size, the intercellular spaces are also large. The nuclei of these cells measure about 10x20 $\mu$., and contain either one or two nucleoli. In some sections at least thirty percent of the nuclei of the cells are binucleolate. This doubling of the nucleoli is not explained except perhaps as an indication of vigorous growth.

The parenchyma cells are all packed with starch grains up to the time that teleutospore production begins. At this time the starch begins to disappear from the cells immediately beneath the teleutosporus. This dissolution of the starch progresses gradually basad as the season advances until at the end of the spore-producing period no starch remains in the parenchymatous tissue. Since the dissolution of the starch and the formation of teleutospores begin together it seems logical to assume that the fungus is drawing on this store of starch for the production of spores. Probably an amylase is produced at this time which converts the starch into sugars available for the fungus. For further data on this question see the section on *Haustoria.*

The walls of the parenchyma cells are composed entirely of cellulose, (at least until April 1, as shown by their positive reaction to iodine and sulphuric acid and negative reaction to phloroglucin).

3. *The Fibro-vascular system* is a modified continuation of the fibro-vascular system of the cedar leaf. From or near the base of the cedar apple, where the vascular elements are much contorted, arise many branches, which extend radially almost to the cortex. The simplest fibro-vascular bundle is composed of a bundle of spiral tracheids, a bundle of the bordered pitted vessels characteristic of conifers, and a sheath of much elongated parenchyma cells. The more complex vascular bundles may contain several bundles of tracheids and bordered pitted vessels bound together and surrounded by elongated parenchyma cells.

c. THE FUNGUS MYCELIUM is found occupying a portion of the intercular spaces between the parenchyma cells. The mycelial threads ramify throughout the cedar apple and grow for the most part closely appressed to the walls of the host cells. The branching of this mycelium is peculiar, the tendency of the strands is to grow in straight lines, and there is some indication of anastomosing. (Fig. 5.) The cells of this mycelium are of widely differing lengths and all are binucleate. The septa between cells are very thin and hard to demonstrate with stains, especially when staining for nuclei at the same time. A preparation dark enough to show the mycelial septa is usually too dark to give good definition of the nuclei.

The mycelium is most abundant just inside the cortex of the cedar apple. At the base of the teleutosorus it forms a compact mass, crowding the host cells almost out of existence. At the base of the cedar apple the mycelium is

much contorted, quite large in diameter, occupies all the space between the host cells, and the terminal portions react to stains in a peculiar manner. These portions stain darkly with safranin but do not take eosin, while the mycelium in other parts of the cedar apples takes eosin readily, but does not take safranin except in the nuclei.

Small cedar apples attached to twigs have been collected at various seasons and sectioned. These sections all show that the mycelium of the parasite does not extend beyond the base of the infected cedar leaf. It does not grow, indeed, into the twig or out into adjacent leaves, but stops at the base of the cedar needle as sharply as if it had encountered a material barrier. This barrier is no doubt the sap of the cedar tree which inhibits the further downward growth of the mycelium. Why the sap of the cedar twig should prohibit the advance of the mycelium and that of the leaf support its growth is not apparent, but such appears to be the case. The gnarled and crooked condition of the hyphae at the base of the cedar apple and their abnormal staining reactions would indicate that some uncongenial substance exists at that point. The question has been raised, "Cannot the mycelium of the parasite extend itself from a cedar apple into the parent twig, thence into adjacent leaves and produce other cedar apples?" The above evidence which appears quite conclusive, opposes this idea.

Careful observation of cedar apples in this connection has shown that in early summer, about June 20th in most seasons, some of the small cedar apples after shedding their sori once more become active. They put forth new lobes, sometimes as many as six or seven, near the base. These lobes take on the dark green color of actively growing cedar apples and expand rapidly, usually pushing outward the apical portion of the cedar twig, which sometimes becomes bent back parallel to that portion of the twig below the cedar apple. Often the apical portion dies and falls away. The twig then appears to terminate in a many lobed cedar apple. Many of these cedar apples become black and die before autumn and most of them fall off. Rarely one may survive. This is evidence that the mycelium may perennate in the gall but not in the twig.

d. Haustoria.—It has been noticed in the above discussion that the mycelium of the fungus grows for the most part in close contact with the walls of the parenchyma cells. Haustoria which penterate these parenchyma cells are given off from the mycelium in the following manner: a swelling occurs on the mycelium, usually where it is in contact with the wall of the host cell, but sometimes on the free end of a hyphal branch. (Fig. 5.) This swelling becomes closely appressed to the cell wall, and its centre, which stains very darkly with safranin, gradually enlarges. The nuclei of the mycelium in the vicinity are usually abnormally numerous and the septa of the mycelium indistinct or at least very hard to demon-

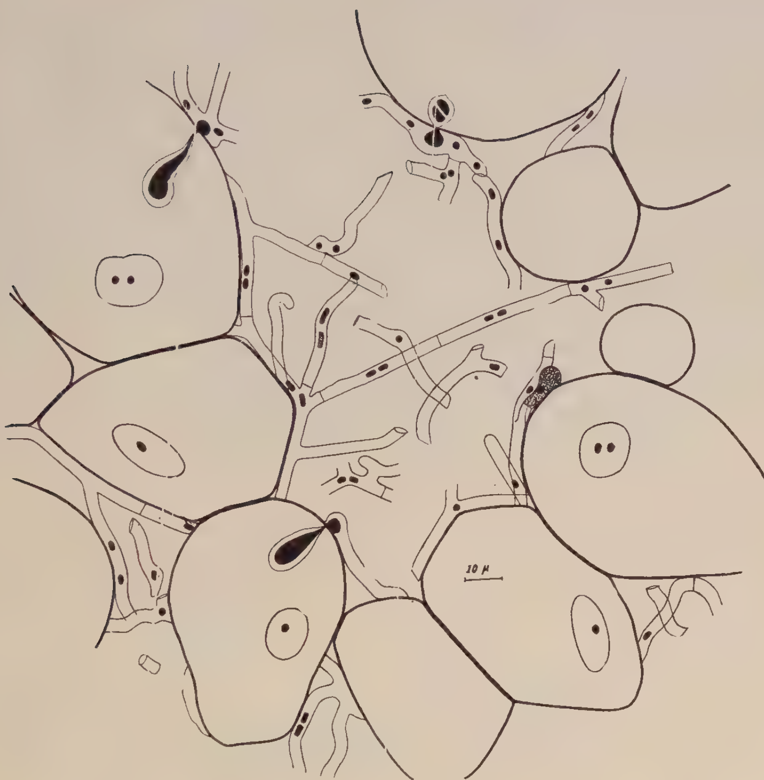


Fig. 5.—Portion of a section of a cedar apple about 5 mm. below a teleutosorus. Note (1) the binucleate intercellular mycelium, (2) the haustoria in various stages of development, (3) the doubling of nucleoli in the nuclei of some of the parenchyma cells of the host. The material from which this drawing was made, was collected March 31.

strate with stains. Finally the wall of the parenchyma cells is broken and a haustorium is pushed into the cell. Haustoria are quite uniformly club shaped, gradually expanding from the narrow portion which traverses the cell wall, and measure 15 to 20 $\mu$. long by 6 to 8 $\mu$. broad. Each consists of a strongly safranin-positive central portion and a non-staining or slightly gentian-violet-positive envelope or capsule. In very young haustoria the safranin positive central portion is divided, as if the haustoria were primarily binucleate. (Fig. 5.) Haustoria have been found only in the parenchyma cells, never in the cortex nor in the spiral tracheids or bordered pitted cells of the vascular bundles.

The time of appearance of the haustoria is most interesting. Sections of cedar apples collected August 30 showed a very few, minute haustoria in the initial stages of development. Material collected on December 7 shows a like condition. A careful search revealed the presence of only a very few

small haustoria no farther developed than those in cedar apples collected August 30.

In sections of cedar apples collected March 28 nearly every host cell was penetrated by a large mature haustorium. Rarely two haustoria have penetrated the walls of the same paranchyma cell. It is evident from these observations that the production and development of haustoria is very slow during the summer, autumn and early winter, and that in March just prior to the production of teleutospores haustoria are developed in great abundance. Up to this time the fungus in the cedar apple grows intercellularly without many haustoria and appears to stimulate the parenchyma of its host to prodigious activity in the multiplication of cells and the storage of starch. Until late winter, i. e., after December, it devotes its activities entirely to an accumulation of plant food, which, as will be seen, is used in the production of teleutospores. At the end of this time, which exactly coincides with the beginning of the formation of teleutosori and the dissolution of the starch of the parenchyma cells, haustoria begin to be produced in great abundance and their production continues with decreasing activity until the teleutospores are mature.

Does the absorption of the starch stored in the parenchyma cells depend on the haustoria? Do the haustoria produce an amylase or in any other way activate this extraction of the starch from the parenchyma cells? Why does the fungus delay the production of haustoria until teleutospore formation begins? are questions which arise, but are as yet unanswered.

The fact that the beginning of teleutospore formation, the extraction of starch from the parenchyma cells and the active production of haustoria are coincident indicates that the haustoria play an important part in the fructification of the fungus. It is probable that by a secretion of amylase these bodies are able to convert the starch of the host cells into sugars which furnish the energy and plant food necessary to carry on the exhaustive process of spore production. Prior to the production of abundant haustoria the fungus, growing slowly and entirely vegetatively, is able no doubt to gain sufficient nourishment by close appression to the walls of the host cells through which it obtains its food by osmosis, or through the few immature haustoria which penetrate a few of the parenchyma cells.

When the period of spore production is near an end in early summer, the cedar leaves on that portion of the twig above the cedar apple and even on many adjoining twigs become colorless, dry up and fall off. The indications are that when the fungus is fruiting it draws all the substance out of nearby leaves by translocation. In some cases where very large cedar apples were watched, the color was extracted at the last fruiting of teleutosori from all leaves on twigs springing out ten cm. or less below the cedar apple. These leaves dried up and shattered off within a week.

Severe infection of cedars and numerous galls often result in the death of the cedar twigs.

e. Teleutosori and spore production.—The first step in the development of a teleutosorus in the cedar apple is the formation of a compact mass of much branched, short celled mycelium just beneath the cortex. This condition first becomes apparent about sixteen months after infection of the cedar leaf, i. e., in the second December of its development. The parenchyma cells of this region are quite small, soon become destitute of starch, and appear to be almost crowded out of existence by the fungous mycelium. A layer of erect rectangular cells arises from this mycelial mass just beneath and perpendicular to the cortex. These cells elongate and their tips take on gradually the characters of the incipient teleutospores. By the use of Fleming's triple stain it is easy to demonstrate the binucleate condition of the cells of these young teleutospores. (Fig. 6.)

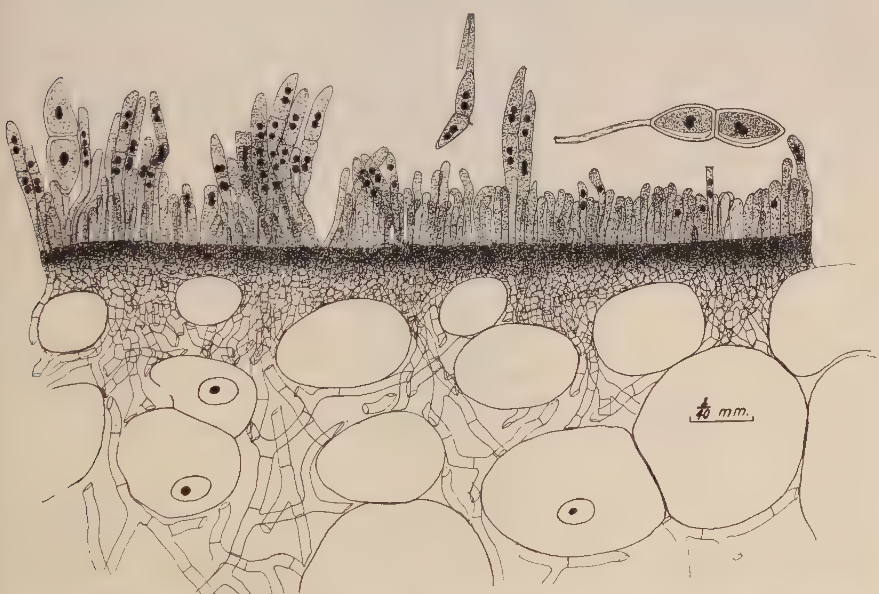


Fig. 6.—Portion of a teleutosorus in February showing the mycelial stroma and the binucleate condition of the cells of young teleutospores.

As will be seen by the accompanying drawing, the prolongation of the rectangular cell which gives rise to a teleutospore at first contains no nuclei, but the nuclei soon migrate upward into it in pairs. The cell wall which divides the teleutospore into two cells then develops simultaneously with the cell wall which divides the teleutospore from the stalk cell. Soon after the young teleutospore becomes recognizable as such the two nuclei of each cell fuse, the walls become much thickened, the stalk cell rapidly elongates into

a pedicel and the spore is thus carried upward. With the increasing pressure of myriads of these rising teleutospores the cortex is finally ruptured and the teleutospore masses project themselves as tentacles. (Fig. 7.) This condition is attained usually about the last of March.

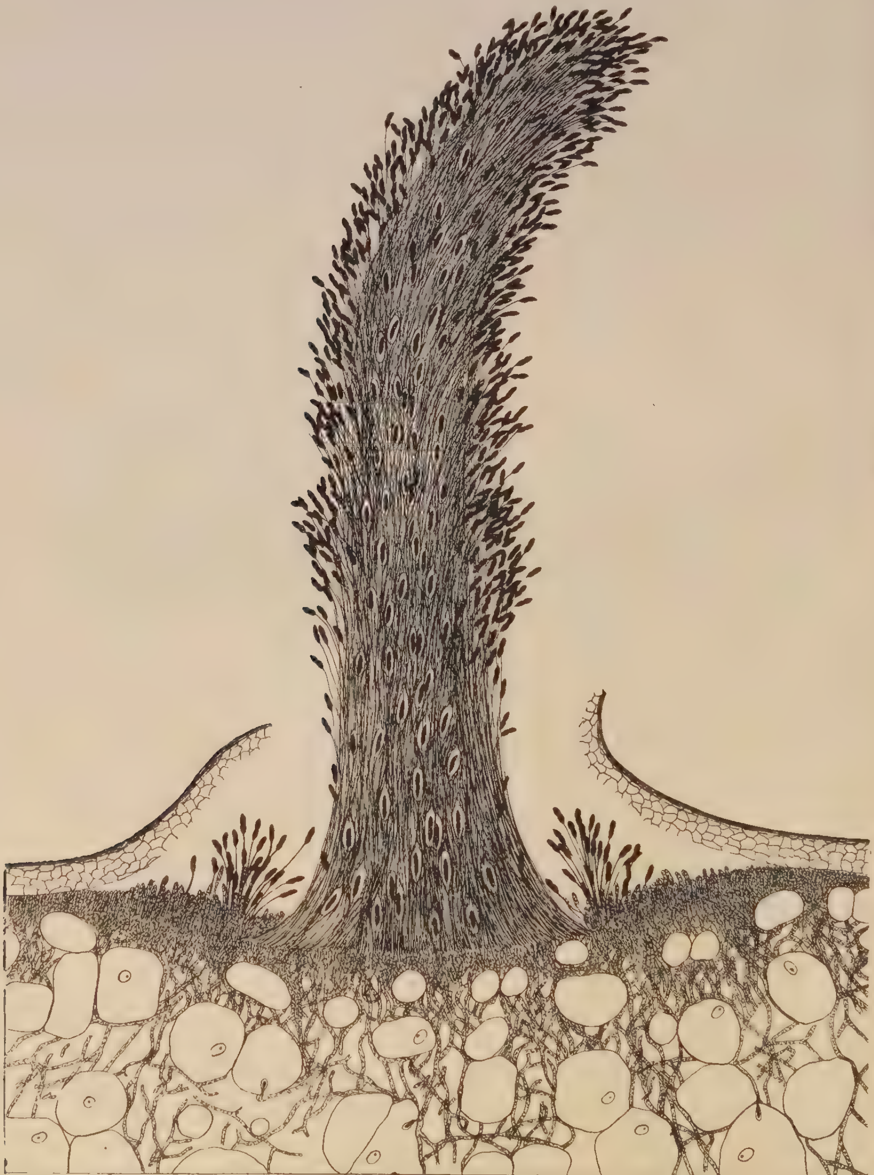

Fig. 7.—Longitudinal section of a partly gelatinous teleutosorus after the first extrusion of tentacles in April.

The manner in which the teleutospores are continously produced is somewhat interesting. As soon as the teleutospore attains its full size the slender pedicel lengthens and carries the spore upward out of the way of those forming later. In this way space is economized and the maximum number of spores produced on a given area.

The teleutospore cell which is binucleate in its earliest stages becomes uninucleate just prior to germination, when it gives rise to four sporidia, each of which is uninucleate. This phenomenon is known as vegetative nuclear division and is presumably accompanied by a reduction of the chromosomes. The transition from the sporophyte to the gametophyte generation thus takes place with germination of the teleutospore.

f. The germination of teleutospores.—During every considerable rain from about April 1 until June 1 the teleutosori become saturated with water. They swell to an enormous size and become highly gelatinous. The teleutospores germinate in situ and produce a promycelium from each cell. The promycelium is cut by septa into five cells, a sterile stalk cell which receives none of the spore protoplasm and four basidial cells, each of which

Fig. 8.—Teleutospores and their germination. From left to right—a typical mature teleutospore, a young teleutospore in which large oil drops are still present, a one celled teleutospore, typical germination of a teleutospore, and a teleutospore showing abnormalities in the mode of germination, viz., swelling of two germpits and production of germ tubes directly from promycelial cells.

bears a sporidium on a short basidium. Thus each normal teleutospore gives rise to eight sporidia. On drying down of the tentacles these sporidia are set free.

The teleutospores on the outside of the tentacle germinate first and shrivel away. Those on the interior of the tentacle then come to the surface and germinate in their turn.

Numerous experiments have been conducted to determine the conditions most suitable for the germination of teleutospores. Abundant moisture is absolutely necessary. A teleutospore must be kept covered with a good film of water or be suspended in water for at least four hours at optimum temperatures. At the temperatures other than optimun germination is slower. To determine the relations of temperature to teleutospore germination a large number of tests were made. The spores suspended in distilled water were incubated at various temperatures. The average results of these tests are given below.

*Germination of Teleutospores.*

| Temperature | Percent germinated |
|---|---|
| 8° C. | 0 |
| 10° C. | 0 |
| 11.5° C. | 5 |
| 13° C. | 71 |
| 15° C. | 98 |
| 16.5° C. | 85 |
| 18° C. | 55 |
| 22-24° C. | 25 |
| 25-28° C. | 25 |
| 29° C. | 2 |
| 30° C. and above | 0 |
| 32° C. and above | 0 |

The optimum temperature for teleutospore germination is 15° C., the maximum 29° C., the minimum about 11° C. The upper thermal death point is 30° C. Spores subjected to this temperature for five hours and then incubated at the optimum temperature showed no vitality. The lower thermal death point has not been determined but it is much below freezing. On the night of April 8, 1914, the temperature went down to –6° C., 80 percent of the teleutospores collected the next morning germinated. In another test a gelatinous cedar apple was frozen in a block of ice. After the melting away of the ice 80 percent of these teleutospores germinated.

Very few sporidia were produced above 20° C. and none above 24° C. Above this point the teleutospores put forth promycelia which never became septate nor produced sporidia. The simple germ tube soon became vacuolate and lifeless. The optimum temperature for germination, it will be noticed, is very low, notably lower than for most fungous spores. There is a remarkable concordance between the optimum germinating temperature and

the actual temperatures which prevail during the rains which gelatinize the cedar apples in the field.

An examination of cedar apples collected at Blacksburg, Virginia, on March 27, 1914, showed them to contain a rather large number, perhaps one percent, of single-celled teleutospores. These teleutospores which

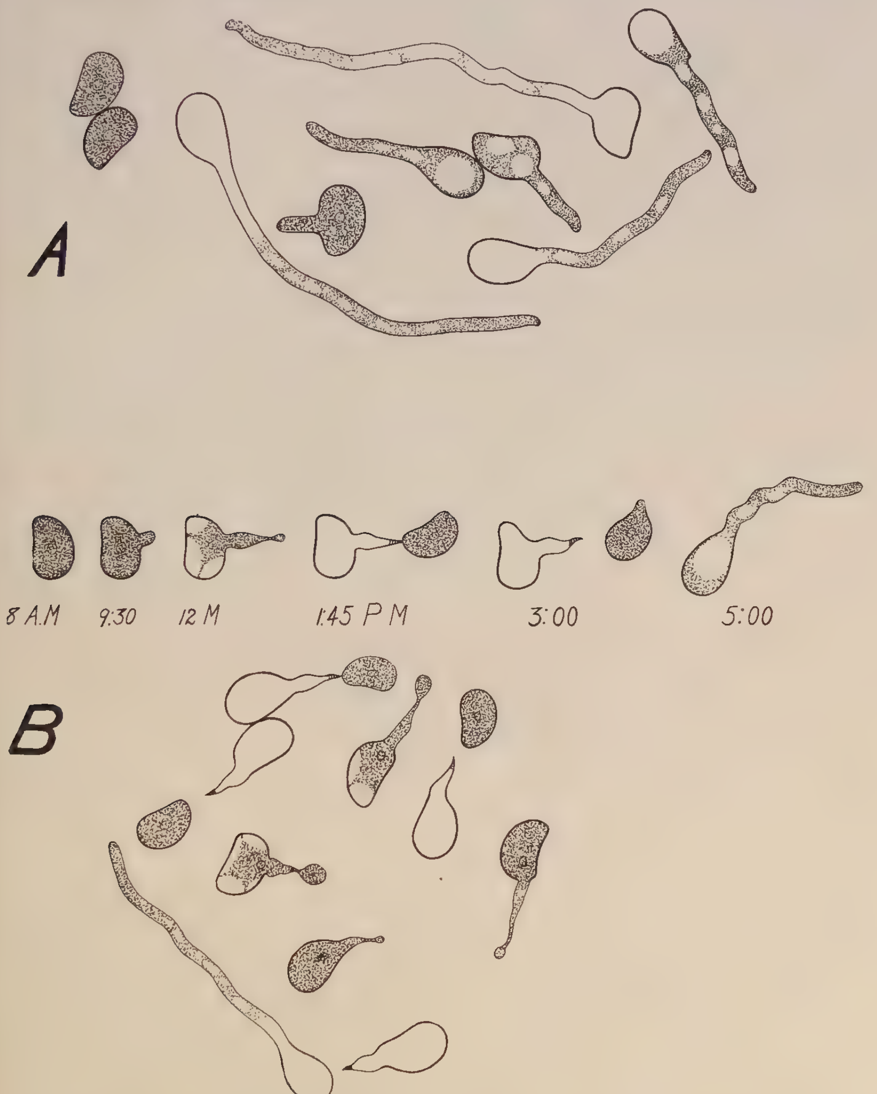


Fig. 9.—Sporidia and their germination. (A) Sporidia germinating directly in hanging drop at 18° C. after being dried over night. (B) Sporidia germinating indirectly in hanging drop at 13° C. Secondary sporidia are produced, which in turn give rise to germ tubes. These sporidia were kept constantly moist from the time they were produced.

germinate, sometimes from the side, sometimes from the base, but usually from the apex, produced normal promycelia and sporidia.

An attempt was made to germinate normal teleutospores and sporidia in an atmosphere of carbon dioxide. The spores were suspended in water and incubated for five hours at 18 to 22° C. in an atmosphere of carbon dioxide. No germination took place. They were then incubated for twelve hours in air. 75 percent of the teleutospores germinated and about 50 percent of the sporidia. After twenty-four hours exposure to carbon dioxide, sporidia would not germinate under optimum conditions. Oxygen is apparently essential for the germination of teleutospores and sporidia.

g. THE GERMINATION OF SPORIDIA.—Germination tests similar to those with teleutospores were conducted with sporidia. Under the conditions which usually prevail in ordinary laboratory tests for spore germination the sporidia of the fungus in question germinate with the simple production of a germ tube. (Fig. 9, A.) But often under natural field conditions and under like conditions artifically produced in the laboratory a totally different type of germination takes place. (Fig. 9 B.) The details of this process have been presented in a previous paper.[1]

In the spring of 1913, the production of secondary spores by the germinating sporidia of *Gymnosporangium juniperi-virginianae* was first observed. These spores have been designated "secondary" sporidia to distinguish them from the primary sporidia from which they originate. The following observations upon the development of these spores have been made.

On March 28, 1913, some "cedar apples" were gathered and placed in a moist chamber. The following day highly gelatinous tentacles had been put forth and teleutospores in abundance were secured. Some of these teleutospores were placed in hanging drops of water and examined from time to time. They germinated in the ordinary way with the production of four sporidia on the promycelium from each teleutospore cell.

On March 30, it was noticed that some of these sporidia had germinated and instead of producing vegetative hyphae had each produced on a short sterigma a secondary spore, identical in shape, color, and markings with the primary spore, but slightly smaller in size.

This aroused suspicion and more hanging drops were prepared. The production of secondary sporidia was observed in all cases. On April 10 the following note was made:

Some Van Tieghem cells were prepared with distilled water and inoculated with sporidia from cedar apples made gelatinous in a moist chamber. One of these showing a good field was clamped under the microscope and watched all day. Each sporidium germinated as follows: A bud-like proc-

[1]Crabill, C. H. Phytopathology. 3:282. 1913.

ess was put forth at some point on the sporidium wall. There seemed to be no definite place to put forth this bud. The bud elongated and the protoplasmic contents of the sporidium extended into it. After growing about 10-25 microns long the bud became pointed and tipped with a little globular body which swelled rapidly and became a secondary sporidium into which the contents of the primary sporidium flowed, leaving the latter empty and hyaline. The secondary sporidium thus produced remained attached to the old sporidium in one case only about an hour. Usually they remain attached much longer. The process on which the secondary sporidium is borne is virtually a sterigma identical in appearance with those on which the sporidia of the promycelium are borne. The secondary sporidia then germinated, producing vegetative hyphae, into which the protoplasm flowed. (Fig. 9, B.) Beyond this no growth took place and the spore and mycelium finally collapsed.

Observations were continued throughout the period of spore production by the cedar apples and almost invariably the production of secondary sporidia took place abundantly.

In two instances, however, April 16 and 19, a large percentage of the sporidia set for germination in hanging drops produced vegetative hyphae directly, without the intermediary production of secondary sporidia. The sporidia used for these two germination tests were secured from cedar apples which were drying after having become gelatinous in a moist chamber.

Later in the season when the warm rains began, it was noticed that when several consecutive days of rain occurred and the cedar apples were kept moist for a long time, the teleutospores germinated with the production of sporidia, which in turn produced secondary sporidia abundantly in situ. When, on the other hand, a shower was followed by sunshine or wind and the cedar apples dried up rapidly, primary sporidia were produced in abundance and shed as soon as dry. If these dry primary sporidia were collected and placed in water they germinated, some producing germ tubes directly and some producing secondary sporidia.

As a result of the investigations in 1913 the following statement was made: "The indications are that, when kept continually moist from the time of production, the primary sporidia will produce secondary sporidia and that, when the primary sporidium becomes dry immediately following its production, and subsequently wet, it may germinate either directly or indirectly. The extent of the dryness may be the determining factor."

Following up the work in 1913, an attempt was made during the spring of 1914 to determine exactly what factors influenced the production of secondary sporidia. Two factors, viz., temperature and moisture were first considered. The results of these tests are presented in two tables. All of the sporidia used in these tests were produced in moist chamber. Those

used in the tests for Table VIII were allowed to become dry before being placed in hanging drop for incubation. On becoming dry the sporidia partially collapse and hang together in orange colored granular masses. If dried for more than twenty-four hours, or even less in bright light, they lose their orange color and will not germinate. The spores used in the tests were produced one day, dried over night out of doors and placed in hanging drop early the next morning.

TABLE VIII.—*Germination of Sporidia Dried Over Night.*

| Temperature | Percent germinated | Percent producing secondary sporidia |
|---|---|---|
| 8° C. | 0 | 0 |
| 13° C. | 98 | 0 |
| 14.5° C. | 95 | 0 |
| 15.5° C. | 90 | 0 |
| 16.5° C. | 25 | 0 |
| 17.5° C. | 8 | 0 |
| 22° C. | 10 | 0 |
| 24° C. | 14 | 0 |
| 26° C. and above | 0 | 0 |

A slight discrepancy exists in the figures for the higher temperatures. This is accounted for by the fact that in some of the cells no spores at all germinated. The reason for this was not apparent.

The sporidia used in the tests for Table IX were kept continually moist, i. e., as soon as they were produced from the teleutospores they were placed in hanging drop and incubated.

TABLE IX.—*Germination of Sporidia Kept Constantly Wet.*

| Temperature | Percent germinated | Percent Producing secondary sporidia |
|---|---|---|
| 13° C. | 98 | 95 |
| 15° C. | 95 | 90 |
| 22-24° C. | 95 | 85 |
| 30° C. | 0 | .. |

A number of times cedar apples kept moist for several days produced abundant secondary sporidia, but the percentage could not be estimated. These tests show the optimum temperature for the germination of sporidia to be 13° C., the maximum about 25° C., the minimum about 10° C. The upper thermal death point is about 30° C., the lower was not determined.

Secondary sporidia are produced only when the sporidia are kept con-

stantly moist from the time of their production until the time of germination. Low temperatures are more favorable to the production of secondary spores even with abundant moisture present than are higher temperatures; 13° C. is the optimum.

If allowed to dry immediately after their production and then subsequently moistened, the sporidia germinate with the production of germ tubes directly.

In the field it has been repeatedly observed during two seasons that when several consecutive days of rain occur, secondary sporidia are produced in abundance and germinate in situ on the cedar apple. In view of the fact that the existence of a germinated spore is precarious and its life short at best, it is evident that many of the sporidia produced during a protracted rainy spell come to naught. Probably nearly all of those produced at the beginning of a long period of gelatinization of a cedar apple die. But so multitudinous are the teleutospores that more than enough are produced near the close of the period to furnish abundant material for the infection of the pomaceous host.

The optimum conditions for the production of sporidia capable of infecting apple trees appear to be as follows: A rain of about twenty-four to thirty-six hours to thoroughly saturate the cedar apple tentacles and then keep them wet for about eight hours; twenty-four hours of wind sufficient to dry down the cedar apple tentacles rapidly and disseminate the spores; an absence of sunshine during dissemination; a rain, mist, or dew to furnish sufficient moisture for the germination of the sporidia after being lodged on the leaves of apple trees.

i. THE LONGEVITY OF SPORIDIA.—Repeated tests have shown that sporidia are short lived. Five or six days is their life limit in an air dry condition. To test the longevity of sporidia the following method was used: Cedar apples made gelatinous in moist chamber or on trees in the field, were suspended over glass microscope slides; as they dried, the sporidia fell and collected as a yellow dust on the slides below. These slides were kept air dry in the laboratory at a temperature of 15-21° C., and the sporidia tested daily in hanging drops of water for germination. The results of two such tests are tabulated below. Each test was made in triplicate and the results are averages.

TABLE X.—*Longevity of Air Dry Sporidia.*

| Sporidia shed | Tested | Germinated |
|---|---|---|
| May 19, 1914 | May 20 | 90 percent |
| | May 25 | 0 percent |
| May 25, 1914 | May 26 | 100 percent |
| | May 27 | 90 percent |
| | May 28 | 90 percent |
| | May 29 | 90 percent |
| | May 30 | 0 percent |
| | May 31 | 0 percent |

In direct sunlight, sporidia are killed in two to five hours. Sporidia produced in moist chamber and 100 percent vital, gave, after twelve hours over calcium chloride, a germination of 0 percent.

Drying is fatal to sporidia even at relatively low temperatures.

j. THE BEHAVIOR OF PROMYCELIA OF RUST FUNGI.—Various writers have shown that the behavior of the promycelia from germinating teleutospores of rust fungi is not at all constant.

Heald[1] has observed in a study of *Gymnosporangium juniperi-virginianae* that instead of producing normal promycelia bearing four sporidia, the promycelial cells sometimes produce hyphae directly. The writers can substantiate this. Again he has noticed that occasionally no promycelium is formed; the sporidia " are produced direct from the side of the teleutospore." This the writers cannot substantiate. Coons[2] has recorded like observations. The junior writer[3] has followed closely the germination of teleutospores of the cedar rust fungus and has demonstrated that when kept continually suspended in water the sporidia almost invariably produce secondary sporidia. Kunkle[4] has brought about the production of a promycelium, sporidia and secondary sporidia by the aecidiospores of *Caeoma nitens*. Klebahn[5] in working with *Puccinia malvacearum* has seen the promycelium break up into four conidia which subsequently put forth sporidia on short sterigmata.

Farlow[6] has recorded the production of four promycelia from a single teleutospore cell. Coons notes only two and that only occasionally. The writers have never seen more than one promycelium develop from a single cell, although a swelling of two germ pits per cell is common.

From the above citations it is evident that the germinating teleutospore may do almost anything. Promycelia seem to be in a state of evolutionary

[1]Heald, F. D. Neb. Agr. Exp. Sta. 22d Ann. Rep't. 1909.
[2]Coons, G. H. Neb. Agr Exp. Sta. 25th Ann. Rep't. 1912.
[3]Crabill, C. H. Phytopathology. 3:282. 1913.
[4]Kunkle, O. Bul. Torrey Bot. Club. 40:361-366. 1913.
[5]Klebahn, H. Zeitschr. Pflanzenkrankheiten. 24:1-32. 1914.
[6]Farlow, W. G. Bot. Gaz. 11:234-241. 1886.

unstable equilibrium. The smallest variations in temperature, moisture or other environmental factors may bring on notable departures from the normal type of sporulation.

## IV.—THE DEVELOPMENT OF THE FUNGUS ON APPLE FOLIAGE.

### 1. Conditions which Affect the Infection of Apple Foliage.

a. AGE OF THE LEAF.—Many observations of rust infected trees showed that the process of infection is not a continuous one. The twigs often show a distinct zonation when the position of rust infected leaves is considered. It is not probable that such a condition would be found if the dissemination of sporidia from the cedar trees were continuous, even though leaves remain susceptible, since each new crop of sporidia could infect all leaves unfolded at the time. Opportunity for observing this condition is most favorable when a drought prevents the fungus on the cedar tree from producing sporidia for a period of several weeks. Water sprouts, since they begin to grow at no uniform time but usually later than the twigs, do not usually show such distinct zones of infection. As a rule the infected leaves on them are nearer the base than on twigs on the same trees.

The season of 1911 afforded an almost ideal opportunity for making such an observation, especially upon the foliage of the York Imperial trees.

Fig. 10.—York Imperial leaves infected with the cedar rust disease. This appearance is common on diseased trees after July 1st.

The trees of this variety put forth very few leaves until the blooming period is over. In 1911 the variety in question bloomed May 8th to 13th. No rains of sufficient magnitude to produce sporidia occurred at Middletown from the time that foliage leaves appeared until the evening of May 31.

The effect of this condition is shown by the figures in Table XI, which gives the amount and distribution of infection upon leaves produced in that season.

The method of presenting these data is somewhat different from any hitherto employed, and since other tables will be presented in the preparation of which the same method was used, a word of explanation may be given before proceeding further.

In obtaining the figures a number of normal twigs were selected so that all the leaves of that season's growth (i. e., on the summer wood) could be examined for rust spots. In each case this gave a series of leaves, the first of which appeared as soon as the trees began to develop and the remaining came out in acropetal succession until about July 1st. Beginning at the base of the season's growth the number of infections upon the first (oldest) leaf were ascertained, then the rust infections upon the other leaves in serial order, from base to apex, were ascertained and recorded. This gives a record of both the absolute and relative amount of infection for a season. Inaccuracy of results depends either upon the use of too few twigs or upon improper choice of twigs. In our work the records were taken after the middle of July and at least five twigs on different sides of the trees were selected as material.

The data given in Table XI show the method of counting the infections, as well as the distribution of the same on the leaves of that year's growth.

TABLE XI.—*Number and Distribution of Cedar Rust Infections upon an Unsprayed York Imperial Apple Tree. McDonald Orchard Season 1911.*

| | Number of cedar rust infections upon leaves in serial order. Leaf numbers | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | Total |
| Twig I | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 34 | 30 | 16 | 30 | 7 | 0 | 131 |
| Twig II | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 12 | 28 | 42 | 11 | 1 | 0 | 0 | 0 | 0 | 114 |
| Twig III | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 5 | 17 | 36 | 24 | 28 | 3 | 0 | 0 | 0 | 0 | 114 |
| Twig IV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 15 | 13 | 34 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 70 |
| Twig V | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 29 | 20 | 4 | 26 | 16 | 0 | 0 | 0 | 0 | 97 |
| Twig VI | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 23 | 39 | 40 | 22 | 14 | 0 | 0 | 0 | 0 | 0 | 140 |
| Total ...... | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 64 | 110 | 158 | 111 | 113 | 50 | 16 | 30 | 7 | 0 | 666 |

The figures show very strikingly the fact that apple leaves are only susceptible to infection with cedar rust during their early stages. The first nine leaves on these twigs are shown to have only seven of the 666 infections produced by the fungus. By the time the maximum infection was taking place these early leaves were beyond the susceptible period and escaped infections.

Fig. 11.—Slightly enlarged photograph of the lower surface of an infected apple leaf, showing the open "cluster cups" from which the aecidiospores are disseminated.

Our weather records show that during the month of May, 1911, there were no rains of sufficient amount to gelatinize the teleutospore tendrils on the cedar apples and consequently no sporidia were produced. It is true that sporidia had been previously produced, but, having no apple foliage to infect, they came to naught. The first nine leaves which developed on the apple trees passed the susceptible stage before any sporidia

had access to them. The sudden increase in the amount of infection when the tenth leaf is reached is due to the fact that rain fell on the evening of May 31 and caused the cedar apples to put forth an abundance of gelatinous tendrils, on which a large crop of sporidia were produced on the ensuing day. These sporidia infected the five or six leaves which had been unfolded just prior to June 1. Another rainless period followed and the leaves subsequently unfolded remained uninfected, as shown by the data in the table.

Penetration of the germ tubes takes place through the dorsal epidermis of the leaf and it therefore seems probable that the increasing thickness of cell walls and cuticle is the factor which determines the period of possible infection.

b. Weather conditions.—From the considerations presented in the foregoing paragraphs as well as in other sections, it becomes evident that weather conditions determine to a great extent the amount of infection which the apple foliage receives. As has just been indicated, leaves 10 to 16 on the twigs examined received their infection following a rain which fell on the evening of May 31 and caused the production of sporidia which were liberated as soon as the tendrils had dried somewhat.

In the case of this fungous disease there is a two-fold necessity for the presence of water—1st, for the production of fresh sporidia and 2d, for the germination of the sporidia after they have reached the apple foliage.

After careful observation of the situation for several years, we find no evidence to support Waite's assumption that there are two strains of the cedar rust fungus,[1] one of which possesses more virulence than the other. It is true, as he stated, that the years 1911 and 1913 saw comparatively light attacks of the disease in the lower Shenandoah Valley, but the same was true to an even greater degree of the year 1914. We believe that the amount of infection can and is to be explained by the climatic conditions of the spring months of those respective years. The year 1911 saw a severe drouth in the month of May, during which time the most of the apple foliage passed through the susceptible period. In 1914 a drouth extended from early May to mid-September. Under such conditions no extensive infection could occur. The year 1912 saw a severe infection of apple foliage, as Waite states. In 1913 the infection was not so severe, because the cedar galls which furnished the sporidia were the result of cedar tree infection occurring in the autumn of 1911, a season in which there were relatively few aecidiospores produced, and therefore comparatively few infections.

We conclude that the chief factor determining the amount of infection of susceptible leaves is the amount of moisture present, and that this is

[1]Waite, M. B. Va. Hort. Soc. Ann. Rep't. 18:43-46. 1913.

necessarily modified by the numbers of sporidia coming from the red cedar trees.

c. Relation to fruit production.—In 1912 a striking difference was observed between the amount of cedar rust infection upon trees with a light crop of fruit and trees having a heavy crop. The trees bearing a heavy crop were less infected by the rust than those bearing a light crop or no crop at all. Not one, but many, instances of this relationship were observed in numerous orchards, and the condition was a subject of frequent remark among the farmers. At present we have no explanation satisfactory to ourselves to assign to this condition. It is difficult to state what differences there might be between the foliage of a bearing and of a non-bearing apple tree at the time when infection is taking place, however great might be the physical and chemical differences later in the season.

d. Injuries caused by insects.—It was noticed in 1911 that trees in a general weakened condition as a result of injury to the roots by woolly aphis, (Schizoneura) showed more rust infection of the foliage than uninfected trees of the same variety in the same orchard.

e. The susceptibility of apple varieties.—Apple varieties show a wide range of susceptibility to cedar rust. Some suffer greatly while others are almost immune. Smith *(Smith's Cider)* is perhaps the most susceptible of all varieties studied. Leaves, fruit, petioles and even twigs are seriously diseased. (Fig. 12.) Trees have been almost killed in some cases.

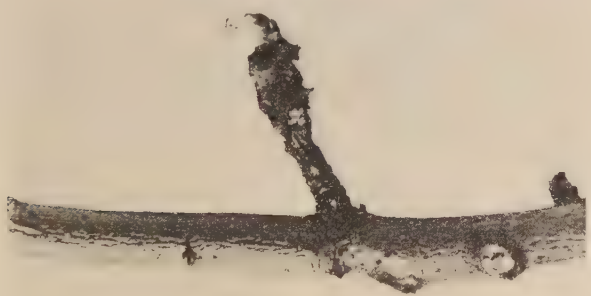

Fig. 12.—Cedar rust on fruit spur of Smith's Cider apple. The leaves, fruit and twigs of this variety are very susceptible to rust and many trees have been almost killed by it.

Among the varieties grown on a large commercial scale, York Imperial, Northern Spy, Jonathan, Bonum, Rome and Ben Davis are very susceptible. Winesap, Stayman Winesap, Arkansas *(Black Twig)*, Yellow Newtown *(Albemarle Pippin)*, Northwestern *(Northwestern Greening)*, and

Grimes *(Grimes Golden,)* are practically immune. Nearly all of the common early summer apples show a high degree of resistance. (For a more complete list of varieties see p. 102.)

Even in the susceptible varieties, the leaves may be infected only while they are young. After they have reached maturity they are immune, possibly due to a thickening of the epidermis, or to the production of some chemical substance repellant to the infecting germ tubes.

A comparative study of the foliage of susceptible and resistant varieties has been made to see if any morphological differences could be detected. Microtome sections of leaves of the same age showed no distinguishable difference in thickness of epidermis, character of stomata or any other property which was constant for any one variety more than another.

Hairiness of leaves was next compared. Hairs perhaps give lodgement for sporidia. It was therefore thought possible that the abundance of hairs might influence the number of infections. Four of the most susceptible varieties were compared with three of the most resistant. Leaves of the same age and position on the twigs were selected. Both upper and lower surfaces were examined. Some of the most resistant ones were as hairy as the most susceptible and vice versa. No correlation could be discerned between susceptibility and hairiness of leaves.

The germination of sporidia in the juice of susceptible and non-susceptible apple leaves was tested. Fresh leaves of Winesap, Arkansas, Grimes, York Imperial and Jonathan were thoroughly washed in tap water and dried. These leaves were all young, measuring one-half to three inches in length. They were hence in a susceptible stage of growth.

Five grams of leaf were ground in a mortar and 10 c. c. of distilled water added. More grinding reduced the leaves to a fine pulp. This was filtered through cotton and used as culture medium for sporidia. The sporidia were taken up from the air dry condition in this apple leaf extract, and hanging drop cultures were prepared. A series of cultures was made in triplicate from each variety of apple leaves. A second series with the above leaf extracts diluted with an equal volume of water was made.

Check cultures were prepared with distilled water.

The average percentage of sporidia germination in the checks was 88. In the first series, germination was entirely inhibited even in extracts of the most susceptible varieties. In the second series where diluted extracts were employed, a very few sporidia germinated feebly in the extract from York Imperial, Jonathan and Blacktwig leaves. There was no germination in extracts of Winesap and Grimes leaves.

f. Nature of resistance.—Of the varieties which suffer least from cedar rust the resistance seems to be of two types. In the first type infection appears to be prevented. Of this type, Grimes and Yellow New-

town are the most notable. Observations have been made on Grimes particularly. Trees of this variety in close proximity to cedars show almost no infection. Those infections which do take place, however, are not stunted but continue to develop throughout the season and produce spermogonia and aecidia just as on susceptible varieties. This leads one to believe that some mechancial, morphological or chemical preventive of infection exists in the epidermis while the palisade and mesophyll tissues are a congenial medium for the growth of the parasite.

In the second type, infection takes place as in the susceptible varieties, the lesions develop until they are about 1 mm. in diameter, and then cease to enlarge. No mature spermogonia or aecidia are produced. Of this type Winesap and Arkansas are good examples. The spots may remain in this condition throughout the season, or they may die. Those which die are often soon infected by saprophytic fungi which enlarge the lesion and produce a Sphaeropsis-like spot. Sections of the abortive rust lesions show that a small amount of hypertrophy has taken place. Immature spermogonia were found in a few. In this type of resistance infection seems to take place without difficulty, but later the development is arrested and the fungus dies out. Our inoculation experiments lend support to this idea.

A somewhat detailed histological study has been made of the aborted rust lesions on Arkansas leaves. About 20 days after infection, rust spots on Arkansas attain a diameter of about 1 mm. Development then ceases, a purple margin forms around the periphery of the yellow spot, which then usually remains in this condition the rest of the summer.

Such spots were collected and killed June 3, 1914. In sections stained with safranin and gentian violet, the wood bundles of the diseased area stand out prominently, having taken the gentian violet much more strongly than the healthy bundles. On the extreme edges of the spot, these dark stained wood bundles are the only evidence of disease. In longitudinal sections of leaves stained with hematoxylin and eosin, the affected wood bundles stand out stained deeply with hematoxylin.

In the central portion of the lesion the spongy parenchyma cells are somewhat hypertrophied, the intercellular spaces obliterated, the upper and lower epidermis intact, and the leaf very little thickened. All the cells are free from green chloroplasts and many of the palisade cells in a state of partial collapse. Passing outward from the centre of the lesion a zone is reached in which the chloroplasts, of the palisade cells especially, are seen to be in a process of disintegration. Outside of this zone all cells appear to be normal except those of the wood bundles as previously mentioned. Perhaps some substance of a toxic nature diffuses from the infected cells and is carried outward for a short distance in the tracheids.

Mycelium is to be found in moderate abundance about the centre of the spot, but only in the hypertrophied spongy parenchyma. The spot extends some distance beyond the mycelium. Even the enlargement of cells seems to extend beyond the ends of the mycelium. No mycelium has been found between the cells of the zone in which chlorophyll grain disintegration is going on.

Rust spots occasionally die out at the centre and then spread rapidly to form a round gray Sphaeropsis-like spot about 3 to 5 mm. in diameter.[1] Such spots collected June 13 were sectioned. The first indication of dying of a spot is the collapse of the palisade cells at the centre of the spot. The upper epidermis becomes flaccid and falls down. The death of the palisade cells is then communicated to the parenchyma cells and lower epidermis. The spot shrinks in thickness and disintegration of the cell walls takes place. In some of these spots, imperfect spermogonia are present. Where such fruiting bodies are numerous the spot is more likely to die out. There is little doubt but that saprophytic fungi are responsible for the spread of rust spots which have died out. They gain entrance through the epidermis by the bursting forth of the immature spermogonia or by actual penetration of the dead epidermis.

The conditions observed may be explained if we assume that a deleterious product of the fungus diffuses into adjacent cells and kills them before the mycelium itself reaches them. The fungus being a strict parasite is unable to grow in these dead cells and accordingly its growth is stopped. There seems to be a paradoxical situation here, inasmuch as the hypersusceptibility of the host is responsible for the death of the parasite.

In 1907 Marryat[1] made a detailed histological study of abortive yellow rust lesions on highly resistant varieties of wheat. She found that the fungus in all cases had entered the host through the stomata and produced a few hyphae. Further progress of the parasite was checked or stopped completely by the death of the host cells attacked. The theory was advanced that resistance in such cases was due " to the production of certain toxins and anti-toxins by host or parasite or both, which are mutually destructive."

A condition somewhat similar in cereals immune to *Puccinia graminis* has been reported by Stakeman at the 1914 meeting of the American Phytopathological Society.[2]

Gradations between this type and very susceptible varieties are to be found. Red Astrachan, for example, is susceptible to infection, but the spots die out when they reach about 2 to 3 mm. in diameter. No aecidia

[1]A similar case has been reported by Heald. Ann. Rep't., Neb. Agr. Exp. Sta. 22:108. 1909.
[1]Marryat, Dorothea. Jour. Agr. Sci. 2:129. 1907.
[2]Stakeman, E. C. Phytopathology. 4:400. 1914.

are ever produced, although a few spermogonia may come to maturity. In such case perhaps the postulated toxin may develop more slowly than in Winesap or Arkansas *(Blacktwig)*.

## 2. Experiments in Infecting Apple Foliage.

Inoculation experiments under conditions which were designed to approach as near the optimum for infection as possible were carried out in 1914.

Twigs were covered in early spring with ordinary white paper bags before any except cluster leaves had unfolded. The leaves which developed on these twigs were later used for inoculation experiments as recorded in each of the following series.

*Series No. 1.*—Two York Imperial trees, ninety meters from cedars, were selected and twigs bagged on May 5.

On May 9, when 4- to 6-twig leaves had unfolded, the bags were removed, the leaves immersed in a sporidia suspension and the bags put back. About 5 c. c. of distilled water were put in each bag to maintain a humid atmosphere for several hours. A layer of cotton was put around the twig at the mouth of the bag to prevent wounding of the bark and to allow an interchange of gases. The results of this experiment, taken July 20th, are presented in Table XII. Leaf numbers are exclusive of cluster leaves.

TABLE XII.—*Series 1. Inoculation Experiments with Leaves of York Imperial Trees. May 9, 1914.*

| Leaf number | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Average number of rust lesions on leaves of 19 inoculated twigs | 1.4 | 3 | 7.4 | 2 | 0.4 | 0.4 | 0 |
| Average number of rust lesions on leaves of 6 control twigs | 1.3 | .3 | 0 | 0 | 0 | 0 | 0 |

A glance at the figures shows that the inoculations were successful. The greatest numbers of infections took place on the second, third and fourth leaves, exclusive of cluster leaves, to unfold on the new wood. The third leaf was in nearly all cases exposed and still so immature as to be susceptible to infection at the time of inoculation. Leaves 5 and 6 in most of the bags unfolded after the inoculation and therefore escaped infection. The infections of leaves 1 and 2 are partly natural, resulting from a dissemination of sporidia which took place following a rain on April 26 and prior to the bagging of the twigs on May 5, but all infections on leaves 3

to 6 may be attributed to the inoculations as the checks testify. Natural infection on leaves 3 to 6 on unbagged twigs was slight. It was almost impossible to find more than six lesions per leaf and very few leaves had more than two or three.

*Series No. 2.*—York Imperial leaves were inoculated and bagged on May 11th. Checks were bagged without inoculation. Plain paper bags were used.

TABLE XIII.—*Series 2. Inoculation Experiments with Leaves of York Imperial Trees. May 11, 1914.*

| Leaf number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Average number of rust lesions on leaves of 16 inoculated twigs | 1.7 | 3.8 | 5.0 | 0.5 | 0 | 0 | 0 | 0 | 0 |
| Average number of rust lesions on leaves of 6 control twigs | 1.2 | 0.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The trees used in this series were 250 meters from cedars. Natural infection on July 20 averaged about one spot per leaf and it was scarcely possible to find more than three spots on one leaf. Leaves 2 and 3 received the greatest infection because they were in the susceptible stage of development.

*Series No. 3.*—Ben Davis leaves, 350 meters from cedars, were inoculated and bagged on May 10. Common paper bags were used.

TABLE XIV.—*Inoculation Experiments with Leaves of Ben Davis Trees. May 10, 1914.*

| Leaf number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Average number of rust lesions on leaves of 13 inoculated twigs | 0.15 | 1.2 | 13.7 | 10.3 | 1 | 0.5 | 0 | 0 |

This set of inoculations was highly successful. No checks were used because these trees were 250 yards in the windward side of the nearest cedars. Natural infection was therefore slight, averaging on July 20 about one spot to four leaves. As many as three spots per leaf were rare. Leaves 3 and 4 were most susceptible at the time of inoculation. Leaves 1, and some of 2, were too old to be infected and on most twigs 5 and 6 had barely begun to unfold.

*Series 4.*—Arkansas leaves, 420 meters from cedars, were bagged May 5, and inoculated May 9. Results were taken July 20.

TABLE XV.—*Inoculation Experiments with Leaves of Arkansas (Blacktwig) Trees. May 9, 1914.*

| Leaf number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Average number of rust lesions on leaves of 12 inoculated twigs | 0.5 | 3.5 | 3.5 | 1.5 | 0.8 | 0 | 0 | 0 |
| Average number of rust lesions on leaves of 4 control twigs | 0.25 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

The spots on the above leaves were minute and had ceased to grow before July 20. They produced no spermogonia or aecidia and some had actually died out. They were nearly all 1 mm. or less in diameter and yellow.

Natural infection on unbagged twigs was about two spots per leaf or less. These results tend to the conclusion that such varieties as Arkansas *(Blacktwig)* owe their resistance to cedar rust, not to an ability to resist infection, but to an ability to overcome the parasite after it has gained entrance to the leaf tissue.

*Series No. 5.*—Ten inoculations of Winesap leaves on May 10th were entirely unsuccessful.

Summarizing the results of the inoculation experiments the following points stand out:

1. The leaves of susceptible varieties may be easily inoculated by this method.

2. Infection takes place only in the presence of abundant moisture.

3. Only young leaves are susceptible.

4. Varieties like Arkansas which produce only abortive rust lesions are decidedly infectable. The failure of such infections to develop has been discussed on a previous page.

### 3. Rate of Leaf Fall in Badly Rusted and Slightly Rusted Orchards.

At some time in August the infected foliage begins to fall from the trees, although it has probably been of no service to the trees for some time prior to its fall. The exact time depends upon the amount of infection and the climatic conditions prevailing; in a dry season the leaves will fall sooner.

An attempt has been made to obtain figures showing the rate of leaf fall in diseased and healthy orchards. No orchard entirely free from rust

was available. One only slightly affected, however, was near at hand and comparison was made of leaf fall in this and a badly diseased orchard. Two branches on each of five trees were tagged in each orchard and the leaves on each counted from time to time. The rate of leaf fall from the time of one count to that of another was calculated in percentage of those present at the beginning of the records.

TABLE XVI.—*Rate of Leaf Fall in Healthy and Diseased Orchards. 1914.*

| Date | Percent healthy | Percent diseased |
|---|---|---|
| August 10-14 | 3.83 | 8.30 |
| August 14-17 | .55 | 4.70 |
| August 17-23 | 4.60 | 3.60 |
| August 23-26 | 2.90 | 2.11 |
| August 26 to September 3 | 1.80 | 1.14 |
| September 3-13 | 1.40 | 3.20 |
| September 13-23 | 5.70 | 7.60 |
| September 23 to October 3 | 2.30 | 8.14 |
| Total | 23.08 | 38.79 |

Table XVI gives an index to the leaf fall rate after August 10th only. Leaf fall began about August 1st and it is safe to say that at least 25 percent of all the leaves on the badly diseased trees fell off before August 10th. Only those leaves which were slightly diseased, and these were many due to the dry spring, remained on the trees. For this reason the above table is incomplete and gives but a partial idea of comparative loss of foliage by diseased and healthy trees for the entire season.

### 4. The Cedar Rust Fungus in the Apple Tissues.

The apple leaf is capable of infection only during its early development. Studies reported in another part of this paper show that a condition of immunity is reached beyond which further infection does not occur. The maximum infection in Virginia usually occurs before the latter part of May. Coons[1] has determined that the sporidia germ tubes penetrate the cuticle of the dorsal surface of the apple leaf and that stomatal infection is not the rule. This is in harmony with the history of other rusts, the germ tubes from uredospores and aecidiospores only penetrating the stomata.

a. THE MYCELIUM of the rust fungus in the apple leaf is much like that in the cedar apple. It is of about the same diameter, ramifies through the leaf in the vicinity of the infection, occupying the intercellular spaces and sending haustoria into the cells of its host. The one great difference be-

[1]Coons, G. H. Neb. Agr. Exp. Sta. Rep't. 25:215. 1912.

tween this mycelium and that found in the cedar apple is that it is uninucleate instead of binucleate, i. e., gametophytic instead of sporophytic. This is easily shown by Flemming's triple stained sections in the mycelium immediately surrounding immature aecidia. The haustoria of this gametophytic mycelium are filamentous, much contorted and exist in the palisade cells and in the spongy parenchyma cells, the latter of which are greatly altered as a result of the stimulation of the parasite.

b. THE HYPERTROPHY of the apple leaf (Fig. 13) is due almost entirely to an excessive enlargement and multiplication of the spongy parenchyma cells. These cells become elongated, stand perpendicular to the upper epidermis, and form what at first glance appears to be a continuation of the palisade layer. The palisade layer, though, always remains intact, and its cells which are much smaller and more uniform in size and shape than those of the hypertrophied spongy parenchyma are only slightly, if at all enlarged. The intercellular spaces of the spongy parenchyma are entirely obliterated. Reynolds'[1] observations on apple leaves parasitized by Gymnosporangium sp. are strictly in accordance with the above statements. Although no haustoria are to be found in any of the epidermal cells a serious injury to the epidermis occurs, especially around spermogonia on the upper surface and over developing aecidia on the lower. This injury consists in a collapse of the epidermal cells. Although no actual rupture of the epidermis is apparent except where the spermogonia and aecidia have broken through, this injury may affect the rate of transpiration in diseased leaves. The stomata of the lower epidermis do not appear to be affected by the parasite. Longitudinal leaf sections show them to be apparently normal. No doubt their functioning is altered by the absence of underlying intercellular spaces and the obliteration of substomatal cavities.

c. SPERMOGONIA.—About one month after infection the apple foliage shows small orange yellow spots on the dorsal surface and on them the spermogonia soon appear. The spermogonia often exude drops of sticky material like honey dew, especially in damp weather. Thaxter[2] reported that flies were observed feeding on the exudate from the spermogonia of this fungus.

The spermogonia are formed immediately under the upper epidermis of the apple leaf. A dense mass of short celled mycelium collects at this point and grows until a size of about 130x120 $\mu$. is attained. As development goes on the mycelium is seen to arrange itself into strands approaching a point at the apex which is to be later the ostiole. No distinct wall is present in the spermogonium, the strands which give rise to the spermatia being continuous with the strands which ramify between the host cells. At

[1]Reynolds, E. S.. Bot. Gaz. 53:365. 1912.
[2]Thaxter, R. Proc. Amer. Acad. Arts and Sci. 22:259. 1887.

maturity the spermogonium ruptures the overlying epidermis, the fungus strands are somewhat protruded, and by abstriction the spermatia are cut off from these strands. The spermatia are thus continuously produced as are the conidia of mildews, until all the mycelium of the spermogonium is used up and only a hollow space remains under the epidermis. The spermatia are ovate to club shaped and measure 2x6 $\mu$. Repeated attempts to germinate these spores have failed.

d. THE AECIDIUM.—The thickened spots on the leaves begin to be distinct about the first of July, and by the middle of that month the aecidia which are borne on the ventral surface begin to break open. If these thickened spots are numerous they cause the apple leaf to be more or less curled.

The aecidia are initiated by the collection of a globular mass of mycelium in the hypertrophied spongy parenchyma immediately beneath the palisade layer. This mass gradually enlarges and elongates toward the lower epidermis as the aecidiospore chains lengthen. Meanwhile the outermost aecidiospores are differentiated to form peridial cells. The developing aecidium finally ruptures the lower epidermis of the leaf and protrudes as a brown papilla. When it has attained a length of about half a millimeter the peridium dehisces, splitting gradually to the base and setting free the aecidiospores. In 1913 the first aecidia opened during the second week in June. As the peridium splits into shreds on dehiscence the shreds curl back giving the open aecidium a stellate appearance. The observations of the writers agree closely with those of Kern,[1] who has given us a description of the structures of the peridium. The peridium is composed of a single layer of cells which forms a covering for the aecidiospore chains within. The peridial cells are virtually modified aecidiospores and are produced by a differentiation of the apical aecidiospores at the top of the aecidium and of whole chains of aecidiospores on the sides of the aecidium. Fromme[2] has recently added to our knowledge of this process in the cup aecidia of several species of Puccinia and Uromyces.

The peridial cells, when viewed from the outside, are diamond shaped fitting together accurately. When viewed from the edge they are oblong with overlapping ends which are firmly joined together. The basal end of each cell laps outside of the upper end of the cell just above it. (Fig. 13.) These joints between cells in the same longitudinal row are quite firm and the cells adhere together tenaciously. The rows of cells however are very loosely joined, and, when the peridium splits, the split always occurs between these rows of cells. These rows of cells will be called *peridial strands*.

The peridial strand is very sensitive to moisture. When it becomes dry it curls outward, opening wide the aecidium. When it is damp it curl

[1]Kern, F. D. Bot. Gaz. 49:445-452. 1910.
[2]Fromme, F. D. Bot. Gaz. 58:1. 1914.

inward, closing the aecidium. Simply breathing on the open aecidia is sufficient to cause them to close immediately. This alternate opening and closing of the aecidia in the presence of moisture or dryness aids in the expulsion of the aecidiospores.

e. THE AECIDIOSPORES.—The chains of aecidiospores arise, according to Blackman, Harper, Christman, Olive, and others, as follows: At the base of the aecidium the end cells of two gametophytic hyphae fuse by a breaking of adjacent cell walls. The nuclei of these cells do not fuse, but lie separate side by side. Then from the binucleate cell thus formed one or more cells bud off, which receive, by conjugate division of the nuclei in the parent cell, two nuclei, and which give rise by conjugate division to chains of binucleate aecidiospores. The conjugation of the ends of the gametophytic hyphae has not been demonstrated, but our sections show plainly the uninucleate mycelium about the aecidium and the binucleate condition of the young aecidiospores and pad cells. The transition from the gametophyte to the sporophyte then takes place with the fusion of the hyphae in pairs at the base of the aecidium.

The aecidiospores are dark brown, minutely pitted, almost spherical bodies. The younger ones are more or less polyhedral, due to the pressure in the aecidium. The walls of these spores are thick and somewhat darker than the granular contents. When placed in hanging drops of water they soon show two or three light spots, apparently the germ pits preparing for the bursting forth of germ tubes. Numerous germination tests however have shown that scarcely any of these spores will germinate in hanging drop, even if a piece of cedar leaf or a little cedar leaf juice be put into the drop.

TABLE XVII.—*Germination of Aecidiospores.*

| Date | Medium | Percent germinated |
|---|---|---|
| August 13, 1912 | Water | 12. |
| August 13, 1912 | Cedar leaf juice | 3 |
| August 13, 1912 | Apple leaf juice | 0 |
| July 15, 1913 | Water | 3 |
| July 18, 1913 | Water | 0 |
| July 22, 1913 | Water | 0 |
| July 23, 1913 | Water | 0 |
| July 24, 1913 | Water | 0 |
| August 30, 1913 | Water and cedar leaf | 0.5 |
| September 14, 1914 | Water | 0 |

Those which do germinate put out a simple, filamentous, vacuolate germ tube which grows to some length. In many instances the ends of these

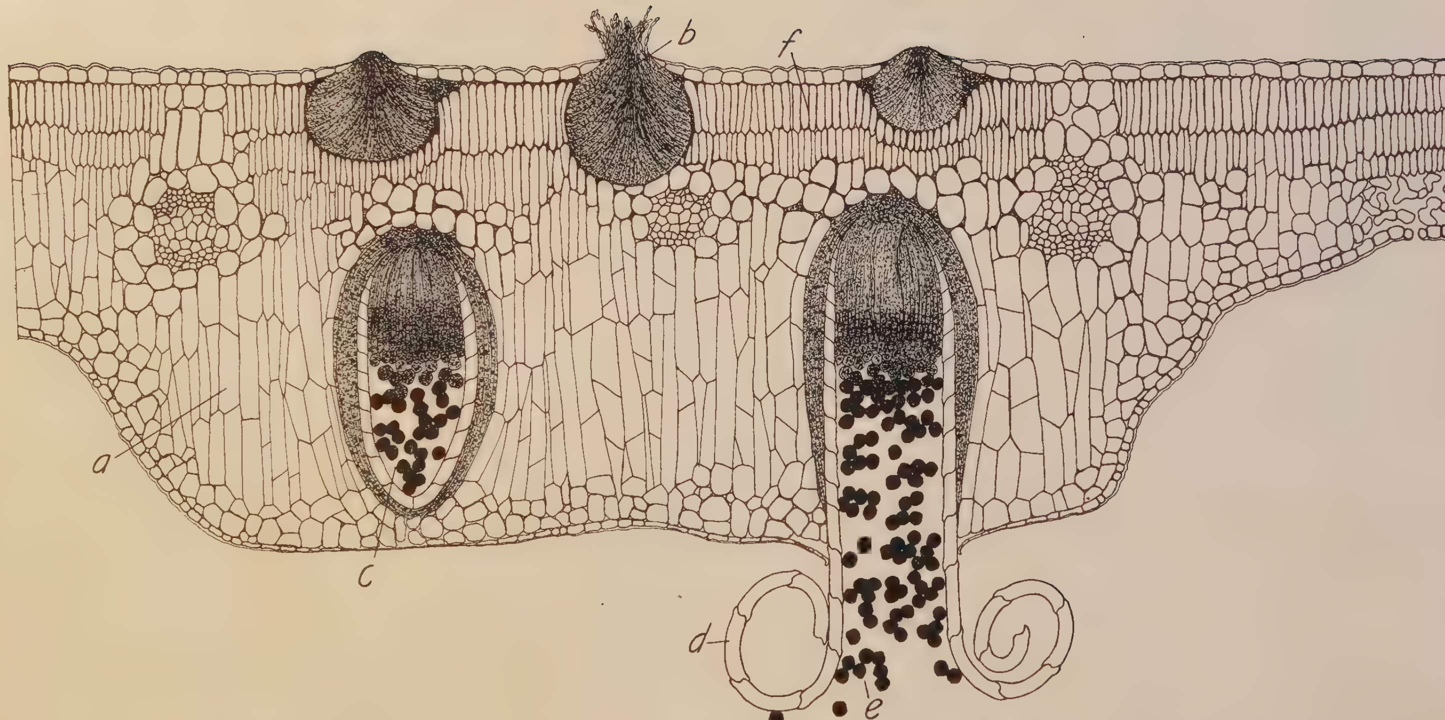


Fig. 13.—Section of a rust lesion on apple leaf. The leaf is greatly thickened by the hypertrophy of the mesophyll tissue, especially the spongy parenchyma (a). The spermogonia (b) are produced immediately under the upper epidermis, the æcidia (c) arise in the hypertrophied parenchyma. (d) peridium, (e) æcidiospores, (f) palisade layer.

germ tubes bulged out into slight enlargements, possibly indicating a feeble attempt to produce a secondary spore.

The inability of these aecidiospores to germinate under what are for most fungi optimum conditions for spore germination appears to throw some light on the time of infection of the cedar by aecidiospores. It will be noticed that at no time during the season was the percentage of germination appreciable. Heald[1] has stated that " The first aecia become mature during the month of July and viable spores are produced in large numbers during this and the two following months. It is during these months that the infection of the cedar takes place, but no visible signs of the infection can be noted. The mycelium apparently remains dormant during the remainder of the season and the winter period, and no ' cedar apple ' is produced, since its production is dependent upon the growth of the cedar which is slightly or entirely inhibited at this period."

In view of the fact that it is impossible to germinate aecidiospores during the season in which they are produced, a more logical explanation of the fact that " cedar apples " do not become apparent until June of the following year is this. Aecidiospores must undergo a resting period or exposure to winter conditions before they will germinate. They are distributed during late summer and autumn, remain in the axils of the cedar leaves where they have lodged and germinate the following spring, giving rise to the young cedar apples which show up about June 10th before any aecidia of the current year's production are open. If this is true the mycelium of *Gymnosporangium juniperi-virginianae* is not perennial as Heald points out, but is strictly annual. Further experiments on this question are under way.

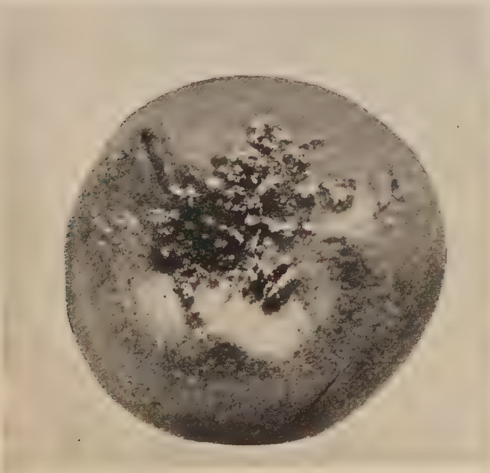

Fig. 14.—An apple affected with cedar rust. Notice that the cluster cups are near the calyx, showing that infection probably entered through a calyx lobe.

### 5. The Cedar Rust on Apple Fruit.

Much of the rust to be found on apple fruit is a result of infection of the calyx lobes at blooming. (Fig. 14.) Many specimens have been collected which bore rust lesions on the side or even on the stem end of the

[1]Heald, F. D. Neb. Agr. Exp. Sta. Rep't. 22:105. 1909.

apple. Jonathan and Ben Davis are particularly susceptible to fruit infection.

The first spermogonia appear on the apples when they are very small, about a centimeter in diameter. Their appearance is identical with that of like bodies on the leaf. Usually, however, a much larger lesion is formed on the fruit than on the leaf, and more spermogonia per lesion. About the time the spermogonia cease to produce spermatia the aecidia break forth from the same lesion. They are arranged in a ring surrounding the area in which spermogonia were produced.

Fig. 15.—Aecidium of cedar rust in the fruit of Jonathan apple.

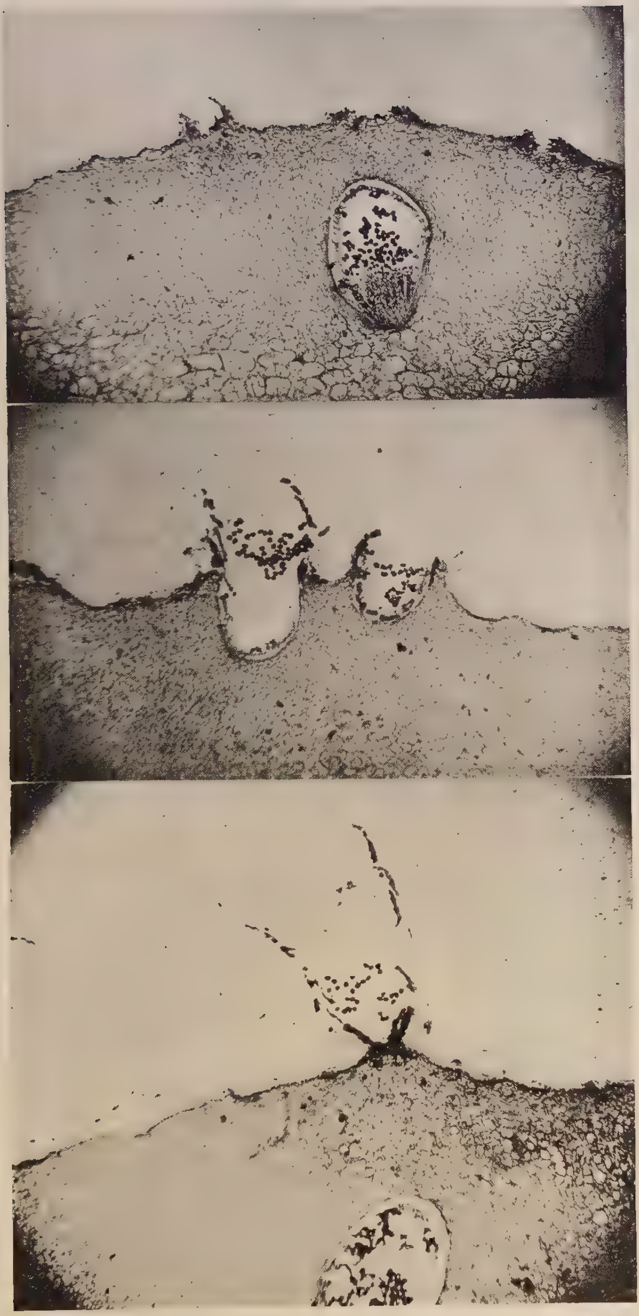

Fig. 16.—Successive stages in the extrusion of aecidia from apple fruit.

The spermogonia are indentical with those produced on the leaf and occur immediately under the epidermis of the fruit.

The aecidia are structurally identical with those on the leaf, but exhibit some interesting relations to the substratum. The tissue of the apple fruit is much hypertrophied as is characteristic of all tissues attacked by this fungus. A layer of oblong cells closely packed and resembling the hypertrophied parenchyma of the leaf is formed by an alteration of the large celled pulp tissue. Deep down in this layer the aecidia begin to form as compact masses of mycelium and, as they develop, gradually migrate toward the surface of the apple. (Fig. 15.) When mature the cuticle of the fruit is ruptured, dehiscence of the peridium takes place and the aecidiospores are expelled. The aecidium is finally pushed bodily out of the fruit, leaving a hole in the skin. (Fig. 16.) All stages of this process are exhibited in sections of rust lesions on Jonathan fruit collected July 25, 1913.

In one instance an aecidium was found which was differently oriented. It had formed about 3 mm. below the surface of the apple and instead of heading in the direction of the surface, it was heading toward the core. Wolf[1] has cited a similar instance in which the aecidia of *Puccinia angustata* opened into the pith of *Lycopus virginicus* instead of opening on the surface of the stem. In this instance the pith had been broken down more or less by the fungus and the aecidia doubtless followed the line of least resistance. With this cedar rust aecidium such is not the case. The line of least resistance would be toward the skin, for at least half an inch of sound apple tissue intervened between it and the core. It will be noticed that on extrusion the basal end of this aecidium would penetrate the skin of the apple first.

## V.—SPOROPHYTE AND GAMETOPHYTE GENERATIONS IN GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE.

The work of Blackman, Christman, Olive and others, has demonstrated that an alternation of generations occurs in many rust fungi. Presumably it occurs in all of them. This alternation of generations may be briefly outlined as follows: The sporidium or basidiospore of a rust fungus germinates with the production of mycelium much like the mycelium of other classes of fungi, i. e., it is thread-like, septate, branching and the cells are uninucleate. The pycnidia or spermogonia produced from this mycelium bear spores which are uninucleate. Later on with the production of the aecidium a doubling of nuclei takes place. The terminal cells of two hyphae at the base of the aecidium come in contact and fuse by a breaking down of the cell walls, but without the fusion of the nuclei. The resulting binucleate fusion cell which may also be called an aecidiospore mother cell, by a series

[1]Wolf, F. A. Mycologia. 5:303. 1913.

of conjugate divisions gives rise to one or more chains of binucleate aecidiospores. These aecidiospores on germination give rise to binucleate mycelium which by conjugate divisions of the paired nuclei, remains binucleate. In the maturing teleutospore a fusion of the two nuclei in each cell takes place, and on germination each cell gives rise by vegetative nuclear division to four uninucleate sporidia. The uninucleate stage of the fungus, i. e., from the sporidium to the aecidium is regarded as the gametophytic generation. In the aecidium the ends of the two gametophytic hyphae, which are to all intents and purposes sexual gametes, unite and initiate the sporophytic generation, which is characterized by binucleate cells, whether vegetative or sporogenous. The sporophytic generation ends with the germination of the teleutospore. The delayed fusion of the paired nuclei after a long series of conjugate divisions, has been called endokaryogamy.

The work here reported substantiates the work of the earlier investigators and adds one more rust, *Gymnosporangium juniperi-virginianae*, to the list of those in which the alternation of generation has been demonstrated.

With Flemming's Triple, Safranin and Hematoxylin, and Iron Alum Hematoxylin, we have been able to show the uninucleate condition of the gametophytic mycelium and spermatia in both the leaves and the fruit of infected apples, and the binucleate condition of the sporophytic mycelium, haustoria, and young teleutospores in the cedar apples. The actual fusion of the paired nuclei in an older teleutospore was one caught by the killing fluid as shown in Fig. 6. (Histological Methods.)

We have been unable to demonstrate the actual fusion of the ends of the gametophytic hyphae at the base of the aecidium but it is easy to see in many sections that the mycelium about the aecidium is uninucleate and that the young aecidiospores and pad cells are binucleate.

## Histological Methods.

Material for sectioning was killed in Chromo-acetic or Gilson's fluid, run through the alcohols, xylols, and imbedded in paraffin and sectioned with a rotary microtome.

Safranin, Gentian violet, Hematoxylin, Eosin and Orange G. were used alone and in various combinations. Heidenhain's Iron Alum Hematoxylin was used for some tissues. Durand's Hematoxylin and Eosin Special was found to be the best combination for demonstrating intercellular mycelium. (Durand, E. J. Phytopathology. 1:129. 1911.) A modification of this method, in which Safranin was employed for nuclei gave the best results in all round work.

After removing the paraffin with xylol the sections are transferred successively to absolute alcohol, 95 percent alcohol and water. After this the following steps are carried out:

1.—Stain over night in Safranin. The same as that used in Flemming's triple, i. e., Safranin 2 g., water 100 c. c., 95 percent alcohol 100 c. c.)

2.—Rinse in water.

3.—Reduce the stain to proper density with 50 percent alcohol.

4. Rinse in water.

5.—Stain for four to ten minutes (depending on thickness of section) in Hema-

toxylin. (Delafields Hematoxylin 1 part, dist. water 3 parts.)

6.—Rinse in a glass of water containing a few drops of concentrated ammonium hydrate.

7.—Dehydrate with four or five pipetfuls of 95 percent alcohol.

8.—Stain for four to ten minutes in Eosin. (Eosin .5 g., 95 percent alcohol 100 c. c.)

9.—Remove the excess of stain from the edges of the slide with a cloth and clear, without washing, in carbol-turpentine. (Carbolic acid crystals, melted, 2 parts, turpentine 3 parts.)

10.—Rinse off the clearer with xylol and mount in balsam.

## VI.—CHEMICAL ANALYSES OF HEALTHY AND DISEASED APPLE LEAVES.

These analyses were made primarily to determine whether there is any correlation between the activities of the leaves and their ash content. Previous studies made on other pathological material showed certain correlations[1] between ash content and teratology.

The analyses were made each year for three years, using leaves collected from healthy and diseased York Imperial trees growing in the same orchard. Each sample of leaves was collected in July, dried in the open air, then ground in a mill and each sample oven-dried just previous to analysis. The analyses were kindly made for us by Dr. W. B. Ellett, chemist of the Experiment Station. The results are presented in Table XVIII.

TABLE XVIII.—*Chemical Analyses of Diseased and Healthy Apple Leaves.*

| | Healthy | | | | Diseased | | | |
|---|---|---|---|---|---|---|---|---|
| | 1911 | 1912 | 1913 | Average | 1911 | 1912 | 1913 | Average |
| Total Ash | 7.57 | 7.25 | 7.05 | 7.29 | 4.54 | 6.95 | 6.90 | 6.13 |
| $K_2O$ | 33.99 | 12.02 | 16.34 | 20.78 | 22.86 | 17.10 | 16.40 | 18.78 |
| $Na_2O$ | 6.62 | 3.95 | 4.05 | 4.87 | 2.72 | 4.86 | 4.28 | 3.95 |
| CaO | 24.99 | 27.90 | 27.94 | 26.94 | 39.47 | 23.99 | 16.07 | 26.51 |
| MgO | 10.27 | 10.04 | 5.82 | 8.71 | 12.70 | 8.71 | 6.30 | 9.24 |
| $P_2O_5$ | 8.29 | 3.80 | 4.74 | 5.61 | 5.37 | 4.31 | 4.58 | 4.75 |
| $SO_3$ | 6.09 | 2.16 | 4.42 | 4.22 | 5.37 | 2.93 | 2.90 | 3.73 |

The ash analyses are difficult of interpretation because of the great fluctuation from one year to another. Wherever marked differences occur in the proportion of any given element in a given year, marked contradiction can be found in the proportion in some other year. However, it may be seen that the total ash is uniformly greater in the healthy leaves. The proportion of other ash constitutents is very variable and there is so little

[1]Reed, H. S. Phytopathology 1:159. 1911.

difference between the three-year averages that we deem it unwise to attempt to draw any conclusions from them.

Analyses to determine the amounts of certain carbohydrates in the healthy and diseased leaves are much more satisfactory, as will be seen from table XIX.

TABLE XIX.—*Relative Amounts of Sugar and Starch in Healthy and Diseased Leaves of York Imperial Apples. Material Collected August, 1913.*

| | Percent | |
|---|---|---|
| | Healthy | Diseased |
| Invert sugar | 2.60 | 1.23 |
| Total sugar | 2.75 | 1.65 |
| Sucrose | .14 | .40 |
| Starch | 4.43 | 2.43 |

An examination of these results shows that the content of starch, invert, and total sugars is higher in the healthy leaves than in those affected with cedar rust. This result is in harmony with the determinations of the photosynthetic activity of the two classes of leaves as measured by the quantity of carbon-dioxide consumed and recorded in another section of this paper. Since the apple leaves do not store any material amount of these substances, it seems safe to assume that the figures give a picture of the carbohydrate manufacture in action. When it is noted that the amount present in the diseased leaves is approximately 50 percent of that in the healthy, it will also be noted that this is nearly the same relation between the average carbon-dioxide consumption of the two kinds of leaves.

The sucrose content in both cases is quite small and the difference cannot be regarded as very significant.

## VII.—THE TRANSPIRATION OF APPLE LEAVES INFECTED WITH GYMNOSPORANGIUM.

This section presents the results of experiments in continuance of our former work.[1]

A survey of the published work upon transpiration discloses few studies of the transpiration of diseased plants, although the assumption is fre-

[1]Reed, H. S., and Cooley, J. S. Bot. Gaz. 55:421. 1913.
Reed, H. S., and Cooley, J. S. Va. Agr. Exp. Sta. Ann. Re'pt. 1911-1912.

quently made that the rate of transpiration is affected by the presence o disease.

Blodgett[1] has reported an observation upon the transpiration of excise branches of *Rubus sp.* infected with *Gymnoconia interstitialis.* In a give period (apparently shorter than 24 hours) the rusted branch absorbe 42 c. c. of water, while a healthy branch possessing an equal number o leaves absorbed only 23 c. c. of water under similar conditions. In spit of the greater amount of water absorbed, the rusted shoot was more o less flaccid, while the healthy shoot maintained a turgid condition. Thi behavior might be regarded as a consequence of the condition produced b the caeoma type of sorus produced by the fungus in question. The ruptur of more or less extensive areas of the ventral epidermis of the leaf obviousl facilitates the evaporation from the spongy parenchyma layers. Possibl other factors connected with the diseased condition may also operate t cause increased transpiration.

While not strictly parallel, it may be proper in this connection to cit results which Burgerstein[2] obtained with the use of dilute solutions o camphor. He found that solutions containing about one part of campho per thousand had an accelerating influence upon most plants investigated Excised shoots, which were previously allowed to become somewhat wilted revived more quickly when placed in camphor water than when placed i distilled water. By weighing the vessels of water in the two cases, it wa shown that transpiration from the shoots went on more rapidly in campho water than in distilled water. That camphor was absorbed by the excise shoots was shown by their pathological condition and death prior to th appearance of any such conditions in the parallel series in distilled wate It seems proper to regard this result as an example of transpiration unde pathological conditions, since the deleterious substances thrown off by fung may act similarly to the camphor.

Results of a somewhat similar import have been reported by one of th authors of this paper, showing that substances like tannic acid and pyro gallol when present in small amounts accelerate transpiration.[3] Smal amounts of oxalic and acetic acids were likewise shown to accelerate trans piration. Since these substances are found as such in plants, it is possibl that they may influence transpiration more or less independently of othe factors.

The studies upon transpiration herein described were conducted upo the apple varieties York Imperial and Ben Davis in orchards near Middle town, Virginia, in 1911 and 1912. All of the trees upon which studie were made were more than eight years old, and, aside from a certai

[1]Blodgett, F. H. Torreya 1:32. 1901.
[2]Burgerstein, A. Verh. Kais. Kon. Zoolog.-Botan. Gesells. Wein. 34:543. 1884.
[3]Reed, H. S. Bot. Gaz. 49:81. 1910.

amount of dwarfing due to continued attacks of cedar rust in one of the orchards, the trees were in good physiological condition.

The time available for making satisfactory studies on transpiration of the diseased leaves was restricted to a period of about six weeks beginning near the middle of July. Before that date the cedar rust had not developed sufficiently to derange seriously, or at least uniformly, the activity of the apple leaves. Subsequent to this period, the fungus had injured or even killed more or less extensive areas in the infected leaves, and, in case of severe infection, the leaves began falling during the latter part of August.

The work here reported was carried out on leaves and twigs on the trees in their normal position. This method was believed to be preferable, since it has been shown by Freeman[1] that actively transpiring shoots do not usually transpire at a normal rate when removed from their own roots.

The data reported in the present paper were obtained by inclosing a few apple leaves in a glass cylinder and absorbing the exhaled water with weighed calcium chloride. The method of carrying on the experiments will be evident from the accompanying sketch of the apparatus (Fig. 17), which is a type modified from that of Freeman *(loc. cit.)* and others.

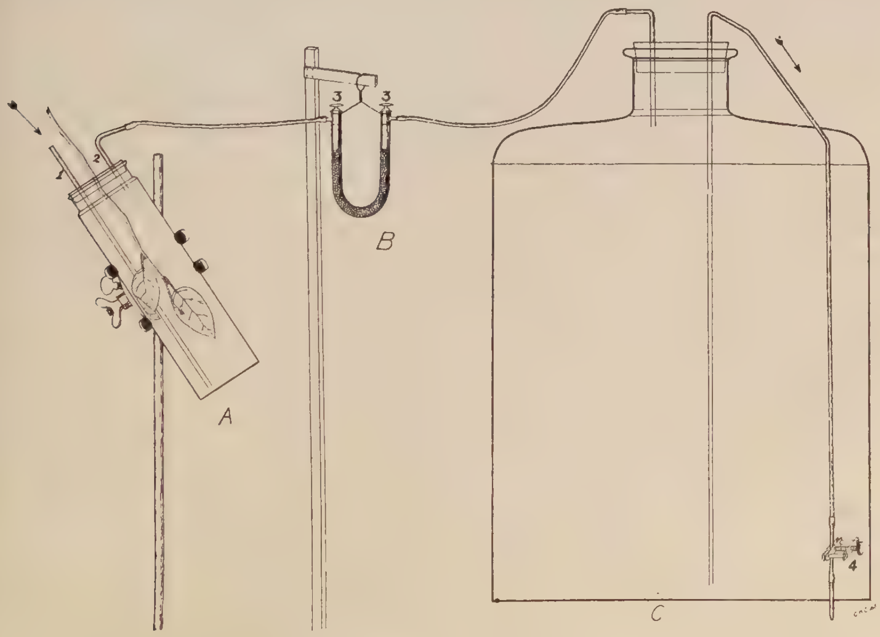


Fig. 17.—Apparatus for measuring transpiration; explained in text.

[1]Freeman, G. F. Bot. Gaz. 46:118. 1908.

In its essentials the apparatus consisted of three parts: a wide mouth glass jar *(A)* which contained the twig under experimentation, a calcium chloride tube *(B)*, and an aspirator *(C)* which drew a known volume of air through the apparatus. The glass jar *(A)* was fitted with a soft rubber stopper which was cut through about three-fourths of its diameter. The opposing surfaces were notched at the centre of the stopper to allow a twig to pass through, but the notch was small enough to insure a tight fit and prevent the passage of air. Two perforations in the stopper allowed glass tubes of 5 mm. diameter to pass. Tube 1, through which the air entered, extended to within 1 cm. of the bottom of the jar; tube 2, through which air left the jar, extended only about 1 cm. inside of the stopper. Tube 2 was connected with rubber tubing to a glass-stoppered calcium chloride tube *(B)*. The calcium chloride tubes were accurately weighed at the laboratory before and after each experiment. The ground stoppers *(3, 3)*, when turned, effectually closed the tubes.

The aspirator for drawing air through the apparatus was a bottle of 19 liters capacity, fitted with a siphon through which the flow could be regulated by means of a screw clamp (4). Three sets of apparatus were constructed and carried in a spring wagon which could be moved from tree to tree as occasion required.

The manner of conducting an experiment was as follows. The apparatus was placed at a tree where direct rays of sunlight would not strike it. A twig bearing leaves suitable for experiment was selected and inserted in the cleft rubber stopper, precautions against bruising or injuring the bark being used. In some cases one or two leaves had to be removed from the twig in order to make a good adjustment. After a few trials it was found that not more than two apple leaves could be used in an experiment with a moderate rate of aspiration; if more were used, water would sometimes collect in the Jar *A*. A thermometer was inserted in the jar with the twig, or suspended close to it. The previously weighed **U**-tube was connected on one side with tube 2 and on the other with the aspirator *C*, which contained 19 liters of water. Care was taken that the temperature of the water used in the aspirator should be very close to that of the air. When the siphon was started, the stoppers *(3, 3)* were turned to allow air to flow through the apparatus. The flow of water could be regulated by means of the screw clamp *(4)* after a little experience, so that the time required to draw out 19 liters should be close to an hour.

The first apparatus was set up with healthy leaves in the jar *A*; another experiment was similarly set up with leaves infected with cedar rust; a third experiment was set up, but with the omission of the jar *A*. These three, each with its own aspirator, were started as nearly simultaneously as pos-

sible, and the temperature kept uniform. The purpose of the third set of apparatus was to determine the amount of moisture in 19 liters of air.

The criticism might justly be made that the evaporating power of the atmosphere was not taken into strict account by this sort of an experiment If the purpose had been to study the conditions or amount of transpiration, such determinations should have been made, but the purpose was to study the comparative transpiration of the healthy and diseased apple leaves, and as such it is believed to fulfil its purpose. Another possibility of error might be found in the vapor pressure from the water in *C*, which would carry back some moisture into the tube *B* and cause the results to be too large. This source of error, however, is obviated by the use of the blank, whose increase in weight was subtracted from each of the accompanying tests.

As soon as the aspirator ceased running, the glass stoppers of the **U**-tubes were closed and the time noted. The leaves were plucked from the twig, placed in a labeled envelope, and taken to the laboratory along with the set of **U**-tubes. After weighing the tubes and computing the gain in weight, the necessary correction was made for the moisture absorbed from sources other than the leaves as registered by the increase in weight of the blank. The outline of the leaves used was carefully traced on paper and the area measured with a planimeter. The results thus obtained were computed and expressed as grams of water transpired per hour per sq. cm.

There were in 1911 and 1912, 52 determinations made upon healthy and diseased leaves of the York Imperial and 26 upon leaves of the Ben Davis. The average ratio of transpiration in the diseased and healthy leaves comes out very nearly the same in each variety of apple studied, 78.3 for the York and 74.2 for the Ben Davis.

The ratios show certain differences if they are grouped according to periods covering different stages in the development of the disease. The first, from July 1 to 15, is a stage in which the fungus is still immature. At that time none of the peridia have broken open, although the thickened cushions are abundant. The second period, from July 17 to 31, marks a time in which the fungus has reached maturity and the leaf of the host begins to exhibit indications of serious injury. The diseased leaves at this time, owing to the expansion of the ventral surfaces by the cluster cups, are rolled toward the dorsal surface. During this second period many peridia open for the liberation of aecidiospores. The third period studied, August 15 to 23, covers a time in which the full effects of the fungus upon its host were very manifest. At that time many of the infected leaves had fallen from the trees, or, if they remained, they had a greater or less proportion of dead tissue. In Table XX the percentages of water transpired in these three periods are given.

TRANSPIRATION BY PERIODS

TABLE XX.—*Ratio of Water Transpired by Diseased Leaves in Daylight. Healthy=100.*

| Dates | York Imperial | Ben Davis |
|---|---|---|
| July 1-16 | 94.7 | 69.1 |
| July 17-31 | 66.5 | 71.3 |
| August 15-23 | 70.7 | 83.4 |
| For entire time of the experiments | 78.3 | 74.2 |

From these figures it appears that in the first period the average unit transpiration of the diseased York leaves was nearly as great as that of the healthy leaves. In the second period the ratio dropped to 66.5, and rose to 70.7 in the third period. The ratios in the case of the Ben Davis leaves did not materially vary from the first to the second periods, but they showed considerable rise in the third period.

The rusted Ben Davis leaves used in the experiments had an average of 7.7 infections per sq. cm.; the York leaves had an average of 5.7 per sq. cm.

Part of the improvement in unit transpiration observed in August is no doubt due to the fact that the most seriously infected leaves (comparable to those used in the foregoing periods) had fallen off, and less seriously infected leaves were used as test objects.

The above data cover results obtained during 1911 and 1912. In 1913 the apparatus used for transpiration tests was somewhat modified and different results were obtained. Referring to Fig 1, the following changes in the apparatus are to be noted: (1) a **U**-tube of $CaCl_2$ was applied to tube 1 to remove all moisture from the air entering the apparatus. (2) a **U**-tube of $CaCl_2$ was placed between B and C to prevent vapor pressure in C from affecting **U**-tube B. (3) Jar A was inclosed in a dark box, excluding all light. The lid of this box was made in two hinged halves notched at the meeting point to allow the passage of the glass tubing and the twig. The box was painted black inside and all joints were light tight. The extra $CaCl_2$ tubes made a blank unnecessary, and consequently only two sets of apparatus were needed, one for diseased and one for healthy leaves. Experiments began July 7 and continued until September 15, aggregating 51 tests.

TABLE XXI.—*Transpiration of Diseased and Healthy York Leaves in Darkness.*

| Date | Mg. water transpired per square cm. per hour | | Ratio of water transpired by diseased leaves. Healthy=100. |
|---|---|---|---|
| | Diseased | Healthy | |
| 1913 | | | |
| July | | | |
| 7 | 2.600 | 3.010 | 86.37 |
| 8 | 4.355 | 2.219 | 196.25 |
| 9 | 2.808 | 2.467 | 113.82 |
| 9 | 5.867 | 4.064 | 144.36 |
| 10 | 3.816 | 4.025 | 94.80 |
| 10 | 4.296 | 3.602 | 119.26 |
| 11 | 3.053 | 1.934 | 157.85 |
| 11 | 5.989 | 3.035 | 197.33 |
| 12 | 5.374 | 2.914 | 184.42 |
| 12 | 4.117 | 2.988 | 137.78 |
| 14 | 3.161 | 2.996 | 105.50 |
| 14 | 4.799 | 7.269 | 66.02 |
| 15 | 4.366 | 7.099 | 61.50 |
| 16 | 3.835 | 3.185 | 120.40 |
| 16 | 5.459 | 5.665 | 96.36 |
| 18 | 5.299 | 5.761 | 91.98 |
| 18 | 9.145 | 6.276 | 145.71 |
| 21 | 3.992 | 4.165 | 95.84 |
| 24 | 5.740 | 6.409 | 89.56 |
| 24 | 3.552 | 4.330 | 82.03 |
| 25 | 3.408 | 3.296 | 103.39 |
| 25 | 4.675 | 3.549 | 131.72 |
| 26 | 3.889 | 3.629 | 107.16 |
| 28 | 6.869 | 3.937 | 174.47 |
| 29 | 7.285 | 4.362 | 167.01 |
| 30 | 6.627 | 5.706 | 116.14 |
| 31 | 2.286 | 2.605 | 87.75 |
| August | | | |
| 1 | 8.302 | 4.076 | 203.68 |
| 2 | 8.370 | 5.818 | 143.86 |
| 4 | 7.920 | 6.721 | 114.43 |
| 5 | 5.897 | 4.536 | 130.00 |
| 6 | 3.598 | 5.322 | 67.60 |
| 7 | 3.565 | 3.990 | 89.34 |
| 8 | 4.754 | 4.460 | 106.59 |
| 9 | 4.016 | 4.444 | 90.36 |
| 13 | 3.338 | 5.692 | 58.64 |
| 13 | 4.871 | 4.074 | 119.56 |
| 14 | 3.820 | 4.432 | 86.19 |
| 15 | 4.213 | 5.344 | 78.83 |
| 18 | 8.546 | 5.982 | 142.86 |
| 19 | 7.632 | 6.409 | 119.08 |
| 20 | 4.783 | 3.205 | 149.23 |
| 25 | 4.491 | 3.295 | 136.29 |
| 26 | 4.094 | 3.845 | 106.47 |
| 27 | 5.476 | 5.219 | 104.92 |
| 28 | 4.874 | 2.938 | 165.89 |
| September | | | |
| 4 | 5.322 | 5.664 | 93.96 |
| 5 | 11.161 | 11.635 | 95.92 |
| 8 | 11.064 | 7.730 | 143.13 |
| 9 | 4.927 | 6.891 | 71.49 |
| 15 | 3,859 | 6.246 | 61.78 |
| Average | 5.2069 | 4.6757 | 111.36 |

Grouping the results presented in Table XXI according to periods covering the three stages in the development of the parasite as was done in Table XX, we have Table XXII.

TABLE XXII.—*Transpiration by Periods.*

| Dates | Average ratio of water transpired by diseased leaves in darkness |
|---|---|
| 1913 | |
| July 7-18 | 111.34 |
| July 21-August 20 | 113.26 |
| August 20-September 15 | 103.36 |
| Entire season | 111.36 |

It is apparent that the modification of the transpiration apparatus brought about a marked change in the results. There are three possible reasons for the difference. (1) the use of drying tubes to remove moisture from the air entering the instruments. There is little likelihood that this change should have much influence, since both instruments were treated alike. (2) the temperature is naturally slightly lower in the jars in darkness than in daylight. (3) the presence or absence of light.

During the season of 1914 an attempt was made to locate the exact cause of the fact that in daylight healthy leaves transpired faster than diseased leaves, while in darkness the process was reversed.

Accordingly, instruments like those used in 1913 were set up without the drying tubes and then with them. The same leaves were used in both tests. Two sets of weighed $CaCl_2$ tubes (B) were prepared. The two instruments were first run simultaneously for an hour on diseased and healthy leaves without the drying tubes attached. The tubes B were then changed for fresh ones, the drying tubes attached and another run of one hour made without removing the twig from the apparatus.

A series of such tests was made. Without the drying tubes there was always a larger yield of moisture in both instruments, but the ratio of moisture transpired by the leaves in the two instruments was the same. This showed that the drying tubes had not altered results.

The temperature factor was next examined. Thermometers were placed in jars A and read at the termination of each test. It was found that the temperature in both jars, whether in daylight or darkness, was always almost the same, seldom differing more than one-half a degree centigrade and never more than one degree. The higher temperature was as likely to occur in one jar as another. We therefore conclude that the temperature

factor as influencing the comparative transpiration of diseased and healthy leaves in the present tests is negligible and may be disregarded.

It is logical to suppose that when the jars are inclosed in a dark box the temperature of the air they contain will be slightly less than when exposed to light. Such was found to be the case. In one instance as much as four degrees centigrade difference was recorded. Seldom though was the difference more than two degrees. But since the decrease in the dark is the same in both instruments this can have no influence on the comparative transpiration of diseased and healthy leaves.

The influence of light and darkness was next tested. Both instruments, with drying tubes, were set simultaneously and run in daylight. A second run was immediately made with the same leaves with jar A inclosed in the dark boxes. The results of these tests appear in Table XXIII.

TABLE XXIII.—*Comparative Transpiration of Diseased and Healthy Leaves in Daylight and Darkness.*

| Date | Diseased | | | Healthy | | |
|---|---|---|---|---|---|---|
| | Area in sq. cm. | Mg. water transpired per sq. cm. per hour | | Area in sq. cm. | Mg. water transpired per sq. cm. per hour | |
| | | Daylight | Darkness | | Daylight | Darkness |
| 1914 | | | | | | |
| July 27 | 52.82 | 6.995 | 6.730 | 65.91 | 5.559 | 4.692 |
| July 30 | 57.64 | 4.299 | 4.175 | 66.11 | 4.881 | 3.134 |
| August 1 | 53.95 | 5.988 | 6.203 | 43.79 | 8.384 | 5.978 |
| August 3 | 60.88 | 4.945 | 3.879 | 65.93 | 4.354 | 1.612 |
| August 5 | 56.63 | 4.993 | 4.398 | 49.21 | 6.258 | 4.322 |
| August 6 | 40.63 | 7.408 | 6.416 | 49.92 | 7.179 | 5.022 |
| August 8 | 41.66 | 7.009 | 7.013 | 36.63 | 9.852 | 8.151 |
| August 13 | 45.02 | 7.250 | 6.645 | 55.21 | 7.413 | 2.604 |
| August 18 | 48.37 | 7.787 | 6.764 | 42.69 | 8.458 | 7.256 |
| | Average | 5.963 | 5.802 | Average | 6.926 | 4.753 |

The above table shows (1) in daylight the healthy leaves transpire more rapidly than the diseased, (2) in darkness they transpire less rapidly than the diseased. (3) The transpiration of healthy leaves varies markedly with the light. (4) That of the diseased leaves is nearly constant whether they are in daylight or darkness.

Darwin[1] found that the rate of transpiration of ivy leaves in light was 136 percent of that in darkness. Of laurel it was 132 percent of that in darkness. The author thinks the increased transpiration in sunlight may be due to warming of the chloroplasts by absorption of radiant energy or that light produces increased permeability of plasmic membranes to water.

[1]Darwin, F. Proc. Roy. Soc. Ser. B. 87:281. 1914.

Lloyd[1] has also found the transpiration of healthy plants greater in daylight than in darkness. He also finds that the ratio of intake to outgo of water is not constant. The stomata are not closely regulatory of the water loss from the leaf and are somewhat ineffectual in maintaining a constant water content. The outgo of the whole plant is greater in daylight than the intake, and at night the reverse is true.

Livingston and Estabrook[2] found in a study of the stomatal movements of five widely different species that the stomata were open in daylight and closed at night. The diffusive capacity of stomata was found to be 8.2 percent at midnight of what it was at 3 P. M. In some cases the diffusive capacity actually reached zero at about midnight.

It is plain from the above citations and the data presented in the present paper that transpiration normally fluctuates within a rather wide range in healthy plants. It is much greater in daylight when the stomata are open than at night when they are closed.

The fact that the transpiration of diseased apple leaves is almost constant whether they are in daylight or darkness leads one to believe that the parasite in some way affects the stomata. It seems possible that the fungus may by means of diffusible toxins or by other means interfere with the response of the stomata, rendering them immovable and unresponsive to the stimulus of light.

A histological study of the rust lesions has shown that the substomatal cavities of the infected portions of the leaf are obliterated, the spongy parenchyma is hypertrophied into closely packed, columnar cells. The stomata themselves may be functionless. At any rate the transpiring surface is much reduced by destruction of the substomatal cavities and the intercellular spaces of the mesophyll tissue.

In leaves only moderately infected, though, this would not account for the whole of the difference between the transpiration rates of diseased and healthy leaves, unless the influence of the parasite is communicated from cell to cell of the host.

Livingston[3] and others have shown that, in certain instances at least, transpiration is a reliable index to growth.

The growth of rusted apple trees is certainly poor and the general vigor and physiological condition bad. The inability of the rusted apple leaves to regulate their transpiration is believed to, in a measure, account for this effect of the parasite upon its host.

[1]Lloyd, F. E. Plant World. 15:1. 1912.
[2]Livingston, B. E. Bull. Tor. Bot. Club. 39:15. 1912.
[3]Livingston, B. E. Bot. Gaz. 40:178. 1905.

## VIII.—THE PHOTOSYNTHESIS OF APPLE LEAVES INFECTED WITH GYMNOSPORANGIUM.

The outcome of experiments upon the photosynthesis of apple leaves diseased with cedar rust has appeared in a recent publication[1] and will not be repeated here. A brief summary of the data set forth seems desirable in order to round up the study. Some additional data obtained during the summer of 1913 will also be presented.

The experiments on photosynthesis were carried out with Ganong photosynthometers.[2] Healthy and diseased leaves were removed from the apple trees and tested at once for carbon dioxide assimilation in the above apparatus. The results were calculated to a unit basis for comparison and recorded as cubic centimeters of carbon dioxide assimilated per hour per square centimeter of leaf.

In brief the average of 20 experiments in 1911 and 1912 show that diseased York Imperial leaves consumed only 56.5 percent as much carbondioxide as healthy leaves from the same trees. Diseased Ben Davis leaves consumed, on the average, 57.6 percent as much carbon dioxide as healthy leaves.

In 1913 as a result of 44 experiments diseased York Imperial apple leaves used 65.3 percent of the amount of carbon dioxide used by healthy leaves.

It has been noticed under a description of cedar rust that the lesions are yellow or orange with red markings. This indicates an absence of chlorophyll in the palisade layers of these lesions. Microscopic examination of sections of rust spots shows no green chloroplasts. The cells of the rust spots appear to contain carotin and erythrophyll, but no chlorophyll. This in itself would account for the decrease in photosynthesis brought about by the presence of the parasite.

The obliteration of substomatal cavities and intercellular spaces in the thickened diseased portion of the leaf and the possible paralysis of the stomata might in some way hinder the exchange of gases. The results with respiration and transpiration tests, however, indicate that at least the stomata remain open wide enough in diseased leaves to allow an increase of water elimination and respiration in darkness.

The fact that the growth of the parasite interferes with the photosynthetic operations of its host lends much to our understanding of the reasons for the poor physiological condition of rust infected trees.

In 1913, studies were continued, using the Ganong photosynthometers and using each time a leaf volume of exactly two cubic centimeters. This

[1] Reed, H. S., and Cooley, J. S. Va. Agr. Exp. Sta. Ann. Rep't. 1911-1912. p. 91.

[2] For description, see Bot. Gaz. 41:209. 1906. Or Ganong, Laboratory course in Plant Physiology, 2d Ed. New York. 1908.

was done by immersing enough leaf tissue in water to raise the water surface two cubic centimeters in a graduated cylinder and cutting the tissue at the water line. Since leaves of the same variety of apple and of approximately the same age were used, the area of leaves used in each test was very nearly similar.

The results of the work in 1913 are presented in tabular form.

TABLE XXIV.—*Effect of Cedar Rust on the Carbon Dioxide Assimilation of York Apple Leaves.*

| No. | Date | Duration of exp't hrs. | Condition of light | Ratio of carbon dioxide assimilation in diseased leaves. Healthy=100. |
|---|---|---|---|---|
| | 1913 | | | |
| 1 | July 8 | 5 | Bright | 114 |
| 2 | " 10 | 4 | " | 62 |
| 3 | " 11 | 4 | " | 70 |
| 4 | " 12 | 6 and 40 m. | " | 81 |
| 5 | " 14 | 6 | Cloudy | 55 |
| 6 | " 16 | 5 | Bright | 50 |
| 7 | " 17 | 6 | Very cloudy | 84 |
| 8 | " 18 | 5 | Bright | 25 |
| 9 | " 19 | 5 | " | 88 |
| 10 | " 19 | 4½ | " | 77 |
| 11 | " 22 | 5 | " | 71 |
| 12 | " 23 | 5 | Cloudy | 43 |
| 13 | " 24 | 6 | Hazy | 62 |
| 14 | " 25 | 5 | Bright | 20 |
| 15 | " 26 | 5 | " | 62 |
| 16 | " 28 | 5 | " | 12 |
| 17 | " 29 | 5 | " | 43 |
| 18 | " 30 | 5 | Hazy | 17 |
| 19 | " 31 | 5 | Bright | 25 |
| 20 | August 2 | 5 | " | 58 |
| 21 | " 4 | 5 | Hazy | 27 |
| 22 | " 5 | 5 | " | 58 |
| 23 | " 6 | 5 | Cloudy | 44 |
| 24 | " 14 | 5 | " | 50 |
| 25 | " 15 | 5 | Bright | 69 |
| 26 | " 18 | 5 | " | 71 |
| 27 | " 19 | 5 | " | 76 |
| 28 | " 20 | 5 | " | 80 |
| 29 | " 21 | 5 | " | 60 |
| 30 | " 25 | 5 | " | 77 |
| 31 | " 26 | 5 | " | 50 |
| 32 | " 27 | 5 | " | 50 |
| 33 | " 28 | 5 | " | 59 |
| 34 | " 29 | 5 | " | 100 |
| 35 | September 1 | 5 | " | 73 |
| 36 | " 3 | 5 | " | 88 |
| 37 | " 4 | 5 | Hazy | 67 |
| 38 | " 5 | 5 | Bright | 112 |
| 39 | " 8 | 5 | " | 136 |
| 40 | " 9 | 5 | " | 111 |
| 41 | " 10 | 5 | " | 100 |
| 42 | " 12 | 4 | Hazy | 52 |
| 43 | " 13 | 6 | " | 72 |
| 44 | " 16 | 5 | Cloudy | 71 |
| | | | Average | 65.3 |

The general average of all tests show that the photosynthesis in the diseased leaves is much depressed, although there is considerable variation in individual cases. In 1913, all the tests were made upon the leaves of the York Imperial variety. A total of 44 tests were made to extend the observations of former years. Having a larger number of tests than formerly enables us to discern the same sort of seasonal variation in photosynthesis as was previously noted in the case of transpiration and reported in Ann. Rep't. for 1911-12, p. 89.

Surveying these figures, it will be seen that the period of lowest photosynthesis in the diseased leaves was between July 18th and August 15th. Before and after that period there appeared to be less injury, as the following figures show:

TABLE XXV.—*Average Ratio of Carbon Dioxide Assimilation in Diseased Leaves.*

| Dates | Ratios |
|---|---|
| July 8-17 | 74 |
| July 18-August 15 | 47 |
| August 18- September 16 | 79 |

Taking the entire season's work and making an average of the 44 tests, it will be seen that the photosynthesis of the diseased leaves was 65.3 per cent of the photosynthesis of healthy leaves, using the amount of carbon dioxide consumed as the measure.

These results showing a decrease of carbon assimilation are confirmed by the chemical analysis of carbohydrate content of leaves reported in another paragraph of this paper.

## IX.—THE RESPIRATION OF APPLE LEAVES INFECTED WITH GYMNOSPORANGIUM.

The literature on the respiration of normal plants is voluminous but that on parasitized plants is scant. Richards[1] has shown that wounded plant tissues respire more rapidly than do normal tissues. His work was done mainly upon potato tubers, the wounding of which consisted in slicing them lengthwise. This was supplemented by like experiments on carrot roots, certain seedlings, leaves and twigs.

Zaleski and Reinhard[2] ground certain seeds, seedlings, etiolated leaves of peas, lupines, wheat, beans, cucurbits, Begonia, and buds of Populus in a

[1]Richards, H. M. Ann. of Bot. 10:530. 1896.
[2]Zaleski, W., and Reinhard, A. Biochem. Ztschr. 35:228. 1911.

mortar and compared the respiration of each with that of the living material. In some cases an increase of respiration was brought about by grinding. In other cases a decrease in respiration was noted.

Irving[1] has increased the respiration of plants with small doses of chloroform, and maintained that increase by repeating the doses at intervals.

Meyer and Deleano[2] report an increase in the process of respiration following the traumatic stimulation due to the separation of leaves from the plants. This reaction period tended to be repeated with each fresh injury to the leaves.

Pavarino[3] in 1908 studied the respiration of grape leaves diseased with Peronospora. He concludes that (1) The parasite, during the first period of the disease, increases both the normal and the intramolecular respiration of the host, (2) In diseased leaves the respiratory quotient is lowered and as a consequence the production of heat is increased, (3) In the diseased leaves the amount of oxidizing ferment is greater than in the corresponding healthy leaves.

The increased respiration is attributed to a superabundance of oxidizing enzymes.

Although some of these investigations are not strictly parallel to the work in hand, they tend to show that injury increases respiration. Parasitism may be regarded as a type of injury. The work here reported was carried on with leaves detached from the tree and inclosed as soon as possible in suitable apparatus for measuring respiration by the output of carbon dioxide.

It will be readily seen then that the tests were made during the period following the stimulation due to removal of the leaves from the trees, but since that stimulation would necessarily be the same in both diseased and healthy leaves, other things being equal, the comparative data set forth in the following pages are believed to afford a fairly reliable index to the respiration of the apple leaves in their normal places on the tree.

During the summer of 1913 respiration was studied by the use of the Ganong respirometer exclusively. (Fig. 18.) This instrument, consists of (1) a glass stoppered oval chamber communicating at one side with a descending graduated cylinder. The capacity of this chamber and cylinder from the zero mark or lowest graduation up is 102 c. c. With 2 c. c. of respiring material in the bulb the air content is thus 100 c. c. (2) A simple ungraduated glass reservoir cylinder, (3) a rubber tube connecting the two. These are so mounted on ordinary laboratory clamps that the reservoir cylinder may be easily slipped up or down.

[1]Irving, Annie A. Ann. Bot. 25:1077. 1911.
[2]Meyer, A., and Deleano, N. T. Ztschr. Bot. 3:657. 1911.
[3]Pavarino, G. L. Atti dell' Instituto Botanico dell' Univ. di Pavia. 11:335. 1908.

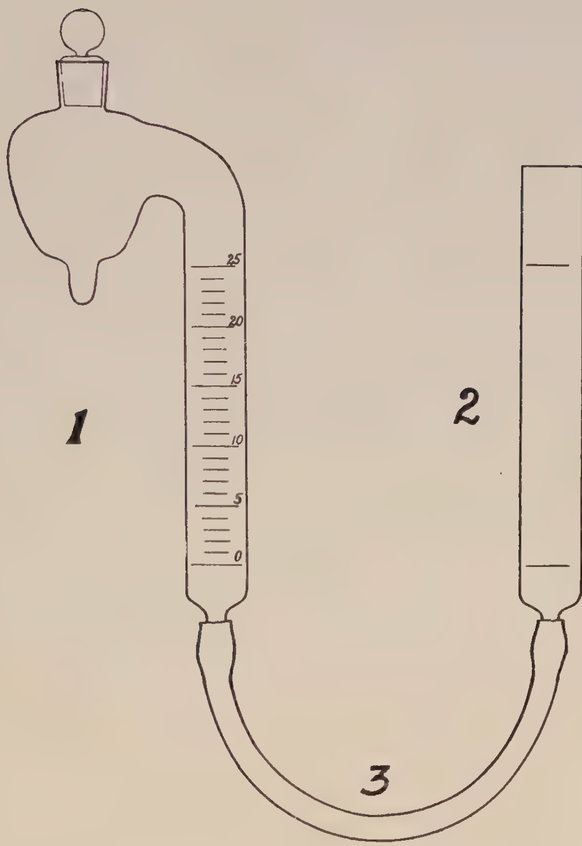


Fig. 18.—Ganong respirometer, the operation of which is explained in the text.

The manipulation of this instrument has been previously described.[1] Healthy and rusted apple leaves were collected at the same time from the same tree and brought immediately into the laboratory in a glass stoppered collecting flask. It was found by experience that a leaf of medium size had a volume of about 1 c. c. Two such leaves were accordingly immersed in a small cylinder of water until 2 c. c. of water were displaced. The petioles were then cut at the surface of the water. The 2 c. c. of leaves were then admitted to the instruments. Two instruments were set up at the same time, one containing healthy leaves and the other rusted leaves. They were immediately inclosed in a dark box where they remained for 12 to 36 hours.

In the absence of light, respiration goes on unmasked by photosynthesis and the gas exchange may be measured quantitatively with a considerable degree of accuracy. In the Ganong respirometer as used in the present

[1]Ganong, W. F. A laboratory course in plant physiology. 2d Ed. p. 129. New York.

studies, the carbon dioxide released is absorbed by a concentrated solution of caustic potash which rises in the graduated cylinder as it absorbs $CO_2$.

At the end of a period arbitrarily established both instruments were read and records made.

It seems desirable to show the relation between the amount and rapidity of respiration and the developmental stages of the parasite. The data are therefore presented in three parts. Part I, from July 3 to July 20, is a period during which the aecidia are still immature. The rust lesions appear as raised thickened cushions, but few peridia are yet apparent, and no aecidiospores have been discharged. Part II, from July 21 to August 20, represents the mature stage of the fungus. It was during this period that aecidiospore production took place, increasing gradually from July 21 to August 5, when it reached a maximum, and then decreasing to the end of the period. By August 20, most of the infected leaves had reached the critical stage of injury from the parasite and had fallen off. Part III, August 20 to September 10, is the period following the production of aecidiospores and the falling of badly infected leaves. The leaves from which these data were obtained were those only moderately infected which still hung on. Results with these leaves are therefore not expected to be so consistent as those of Parts I and II. It is evident from the character of the work that the periods are not clear cut and distinct, but must necessarily overlap slightly.

The data obtained during the summer of 1913 are presented in Table XXVI, which is divided into three parts as above designated.

TABLE XXVI.—*Respiration of Diseased and Healthy York Imperial Leaves. Ganong Respirometer Tests.*

| Date, 1913 | Duration of experiment in hours | Temp. | c. c. of $CO_2$ respired | | c. c. of $CO_2$ respired per c. c. of leaf per hour | | Ratio of $CO_2$ respired by diseased leaves. Healthy=100 |
|---|---|---|---|---|---|---|---|
| | | | Diseased | Healthy | Diseased | Healthy | |
| | | | PART I | | | | |
| July 3 | 24 | 24° C. | 9.0 | 8.0 | .1875 | .1666 | 112.54 |
| 7 | 20.5 | 21° C. | 9.0 | 7.0 | .2195 | .2107 | 181.85 |
| 9 | 23.5 | 16° C. | 5.0 | 6.0 | .2127 | .2553 | 83.31 |
| 10 | 24 | 21° C. | 4.5 | 4.0 | .0937 | .0833 | 112.48 |
| 12 | 24 | 23° C. | 14.0 | 9.5 | .2916 | .1979 | 147.34 |
| 14 | *48 | 22.2° C. | 18.5 | 13.0 | .... | .... | .... |
| 15 | 24 | 23.7° C. | 13.5 | 9.7 | .2812 | .2020 | 139.20 |
| 16 | *48 | 21° C. | 13.5 | 13.5 | .... | .... | .... |
| 17 | 24 | 21° C. | 10.2 | 8.7 | .2125 | .1812 | 117.27 |
| 18 | 24 | 21.6° C. | 8.0 | 6.0 | .1666 | .1250 | 133.28 |
| | | | | Average | .2081 | .1727 | 120.49 |

*A continuation of the preceding experiment. These results are submitted simply to show that the healthy leaves may finally surpass the diseased ones in total $CO_2$ respired. To avoid a partial duplication of results these data are omitted from the columns to be averaged.

PART II

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21 | 24 | 21.6° C. | 11.0 | 7.5 | .2208 | .1562 | 141.35 |
| 23 | 24 | 21.6° C. | 8.0 | 6.0 | .1666 | .1250 | 133.28 |
| 25 | 24 | 21.6° C. | 6.5 | 6.5 | .1354 | .1354 | 100.00 |
| 26 | 24 | 21.6° C. | 4.0 | 9.5 | .0833 | .1954 | 42.09 |
| 29 | 24 | 22.8° C. | 7.0 | 6.5 | .1458 | .1354 | 107.68 |
| 30 | 24 | 22.2° C. | 9.5 | 6.0 | .1979 | .1250 | 158.32 |
| 31 | 24 | 21.6° C. | 5.5 | 5.0 | .1145 | .1041 | 109.99 |
| Aug. | | | | | | | |
| 1 | 24 | 24.4° C. | 6.5 | 6.0 | .1354 | .1250 | 108.32 |
| 2 | 24 | 23.7° C. | 7.0 | 9.0 | .1458 | .1875 | 77.76 |
| 3 | 24 | 22.2° C. | 10.5 | 8.5 | .2187 | .1770 | 123.55 |
| 5 | 24 | 21.6° C. | 6.5 | 6.0 | .1354 | .1250 | 108.32 |
| 6 | 24 | 21.6° C. | 12.5 | 9.5 | .2437 | .1979 | 123.14 |
| 7 | 24 | 21.6° C. | 7.0 | 8.0 | .1458 | .1666 | 87.51 |
| 8 | 24 | 22.7° C. | 5.5 | 5.0 | .1145 | .1041 | 109.99 |
| 9 | 24 | 22.7° C. | 6.0 | 7.0 | .1250 | .1458 | 85.73 |
| 15 | 24 | 21.6° C. | 10.0 | 8.5 | .2083 | .1770 | 117.68 |
| 16 | 24 | 21.6° C. | 9.0 | 3.0 | .1875 | .0625 | 300.00 |
| 20 | 24 | 21.6° C. | 6.6 | 6.5 | .1354 | .1145 | 118.25 |
| | | | | Average | .1588 | .1423 | 111.59 |

PART III

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21 | 24 | 21.1° C. | 5.0 | 5.0 | .1041 | .1041 | 100.00 |
| 26 | 24 | 21.6° C. | 4.0 | 6.0 | .0833 | .1250 | 66.64 |
| 27 | 24 | 22.7° C. | 7.0 | 5.0 | .1458 | .1041 | 140.05 |
| 28 | 24 | 22.2° C. | 7.0 | 5.0 | .1458 | .1041 | 140.05 |
| 29 | 24 | 22.2° C. | 7.0 | 3.0 | .1458 | .0625 | 233.28 |
| 30 | 24 | 22.7° C. | 7.0 | 4.0 | .1458 | .0833 | 175.03 |
| Sept. | | | | | | | |
| 4 | 24 | 22.7° C. | 7.5 | 7.5 | .1562 | .1562 | 100.00 |
| 5 | 24 | 23.3° C. | 4.0 | 7.0 | .0833 | .1458 | 57.13 |
| 9 | 24 | 21.6° C. | 8.5 | 7.5 | .1770 | .1562 | 113.31 |
| 10 | 24 | 17° C. | 7.0 | 7.0 | .1458 | .1458 | 100.00 |
| 11 | 24 | 20° C. | 5.0 | 3.0 | .1041 | .0625 | 166.56 |
| 13 | 24 | 21.1° C. | 10.0 | 9.0 | .2083 | .1875 | 110.90 |
| 16 | 24 | 16.7° C. | 12.0 | 11.0 | .2500 | .2295 | 108.93 |
| 17 | 24 | 21.6° C. | 5.0 | 5.0 | .1041 | .1041 | 100.00 |
| | | | | Average | .1428 | .1264 | 112.97 |
| Average for entire season | | | | | .1699 | .1471 | 115.49 |

In the summer of 1914 the manipulation of the Ganong Respirometer was somewhat modified. Instead of using 2 c. c. of leaves in the respiring chamber, one or two grams of leaves, carefully weighed out, were used. Results were recorded on sheets similar to those used in 1913. Table XXVII presents the results of these tests, divided into three groups as in Table XXVI.

TABLE XXVII.—*Respiration of Diseased and Healthy York Imperial Leaves. Ganong Respirometer Tests.*

| Date, 1914 | Duration of experiment in hours | c. c. of $CO_2$ respired | | c. c. of $CO_2$ respired per gm. of leaf per hour | | Ratio $CO_2$ respired by diseased leaves. Healthy=100 |
|---|---|---|---|---|---|---|
| | | Diseased | Healthy | Diseased | Healthy | |
| | | | PART I | | | |
| July | | | | | | |
| 16 | 48 | 8.0 | 8.0 | .0833 | .0833 | 100.00 |
| 17 | 13 | 8.5 | 7.5 | .3269 | .2884 | 113.34 |
| 18 | 12 | 13.0 | 11.5 | .5416 | .4791 | 113.04 |
| 19 | *24 | 15.5 | 17.0 | .... | .... | ...... |
| 21 | 13 | 12.5 | 9.5 | .4807 | .3653 | 131.59 |
| 21 | 8 | 6.0 | 2.0 | .3750 | .1250 | 300.00 |
| 22 | 12 | 12.0 | 12.0 | .5000 | .5000 | 100.00 |
| 22 | *24 | 16.5 | 17.0 | .... | .... | ...... |
| 23 | 15 | 9.0 | 8.5 | .3000 | .2833 | 105.89 |
| 23 | *24 | 11.0 | 11.0 | .... | .... | ...... |
| 24 | 14¾ | 7.5 | 7.0 | .2542 | .2372 | 107.16 |
| 25 | 15 | 7.0 | 8.0 | .2333 | .2666 | 87.50 |
| 26 | 15 | 10.0 | 7.0 | .3333 | .2333 | 142.86 |
| 27 | 15 | 7.0 | 6.0 | .2333 | .2000 | 116.65 |
| 28 | 16 | 4.0 | 9.5 | .1250 | .2968 | 42.11 |
| 30 | 16 | 9.5 | 12.0 | .2968 | .3750 | 79.14 |
| 30 | *25 | 13.0 | 13.0 | .... | .... | ...... |
| Average | | | | .3141 | .2871 | 109.40 |
| | | | PART II | | | |
| Aug. | | | | | | |
| 1 | 14 | 8.5 | 8.0 | .3035 | .2857 | 106.23 |
| 2 | *39 | 9.0 | 12.0 | .... | .... | ...... |
| 3 | 15 | 10.0 | 8.5 | .3333 | .2833 | 117.64 |
| 5 | 15 | 10.5 | 9.0 | .3500 | .3000 | 116.66 |
| 6 | 15 | 11.5 | 9.0 | .3833 | .3000 | 127.76 |
| 6 | 14 | 11.5 | 9.0 | .4036 | .3166 | 127.47 |
| ** 8 | 9 | 3.5 | 1.5 | .3888 | .1666 | 233.37 |
| 9 | 24 | 9.0 | 2.0 | .3750 | .0833 | 450.18 |
| 10 | 24 | 9.0 | 7.5 | .3750 | .3125 | 120.00 |
| 11 | 15 | 4.0 | 2.5 | .2666 | .1666 | 160.02 |
| 12 | 16 | 6.0 | 5.0 | .3750 | .3125 | 120.00 |
| 13 | 15 | 6.0 | 3.0 | .4000 | .2000 | 200.00 |
| 14 | 15 | 4.0 | 5.5 | .2666 | .3666 | 72.72 |
| 15 | 12 | 6.0 | 3.0 | .5000 | .2500 | 200.00 |
| 16 | 15 | 7.0 | 2.0 | .4666 | .1333 | 350.03 |
| 18 | 15 | 5.5 | 2.0 | .3666 | .1333 | 275.01 |
| 19 | 15 | 5.5 | 4.0 | .3666 | .2666 | 137.50 |
| 20 | 15 | 5.0 | 4.0 | .3333 | .2666 | 125.01 |
| 22 | 15 | 6.0 | 5.5 | .4000 | .3666 | 109.11 |
| 23 | 15 | 6.5 | 7.0 | .4333 | .4666 | 92.86 |
| 24 | 15 | 5.0 | 4.0 | .3333 | .2666 | 125.01 |
| 26 | 20 | 6.0 | 5.0 | .3000 | .2500 | 120.00 |
| 27 | 15 | 5.0 | 5.0 | .3333 | .3333 | 100.00 |
| 29 | 57 | 17.0 | 12.0 | .2982 | .2105 | 141.66 |
| 31 | 11 | 3.0 | 3.0 | .2727 | .2727 | 100.00 |
| Average | | | | .3593 | .2629 | 136.66 |

**Beginning August 8 only one gram of leaf was used instead of two grams as before that date.

PART III

| | | | | | | |
|---|---|---|---|---|---|---|
| Sept. | | | | | | |
| 2 | 12 | 8.0 | 6.0 | .6666 | .5000 | 133.32 |
| 3 | 12 | 10.5 | 8.5 | .8750 | .7083 | 123.53 |
| 6 | 12 | 8.0 | 6.0 | .6666 | .5000 | 133.32 |
| 7 | 24 | 12.0 | 8.0 | .5000 | .3333 | 150.02 |
| 8 | 12 | 5.0 | 5.5 | .4166 | .4583 | 90.90 |
| 9 | 24 | 5.5 | 4.5 | .2291 | .1875 | 122.18 |
| 13 | 12 | 2.5 | 4.0 | .2083 | .3333 | 62.49 |
| 14 | 12 | 6.0 | 5.0 | .5000 | .4166 | 120.01 |
| 15 | 12 | 2.0 | 2.0 | .1666 | .1666 | 100.00 |
| 16 | 12 | 2.5 | 2.0 | .2083 | .1666 | 125.03 |
| 17 | 12 | 3.0 | 2.5 | .2500 | .2083 | 120.01 |
| 17 | 12 | 3.0 | 2.5 | .2500 | .2083 | 120.01 |
| 18 | 12 | 2.0 | 2.0 | .1666 | .1666 | 100.00 |
| 19 | 12 | 3.5 | 3.5 | .2916 | .2916 | 100.00 |
| 19 | 12 | 2.0 | 2.0 | .1666 | .1666 | 100.00 |
| 20 | 36 | 8.0 | 7.0 | .2222 | .1944 | 114.30 |
| 21 | 12 | 4.5 | 4.0 | .3750 | .3333 | 112.51 |
| 22 | 12 | 3.5 | 3.0 | .2916 | .2500 | 116.64 |
| 23 | 12 | 3.0 | 3.0 | .2500 | .2500 | 100.00 |
| 23 | *24 | 4.5 | 5.0 | .... | .... | ...... |
| 24 | 12 | 1.5 | 2.0 | .1250 | .1666 | 75.00 |
| 25 | 24 | 5.0 | 5.0 | .2083 | .2083 | 100.00 |
| 28 | 24 | 5.0 | 5.0 | .2083 | .2083 | 100.00 |
| Oct. | | | | | | |
| 3 | 24 | 5.0 | 4.0 | .2083 | .1666 | 125.03 |
| Average | | | | .3239 | .2865 | 113.05 |
| Average for entire season | | | | .3324 | .2788 | 119.22 |

An examination of the data presented above shows that respiration is more rapid in diseased than in healthy leaves. In tests running for 12 to 15 hours, the diseased leaves almost invariably gave off more carbon dioxide than did the healthy leaves. But, when the test was continued on these same leaves for 24 hours or more, the healthy leaves usually caught up with, or even surpassed, the diseased leaves in total carbon dioxide respired. In the diseased leaves respiration appears to go on very rapidly until all of the starch available for this process is exhausted, while the healthy leaves respire more slowly and exhaust their starch less quickly. From the results of the long runs of the apparatus it seems evident that the healthy leaves contain more food material available for respiration than the diseased leaves.

Respiration of diseased leaves for the second period was 136.66 percent of that of the healthy leaves as against 109.40 percent and 113.05 percent for the first and third periods respectively. This indicates that during the period the fungus is fruiting the respiration of the diseased leaves is markedly increased. In the healthy leaves it will be noticed that the average respiration is actually slightly less in the second than in the first and third periods, while in the diseased leaves it is considerably more. This

appears to be in accordance with the results obtained by Maige.[1] From observations made on many fleshy fungi he found that in the reproductive portion of the plants respiration was much more active than in the vegetative portions.

In addition to and running parallel with the Ganong respirometer tests of 1914, other tests were made with modification of Sachs' apparatus. In this apparatus the leaf is enclosed in a **U**-tube through which a current of $CO_2$-free air is drawn and passes into a standard barium hydrate solution. The carbon dioxide respired by the leaf precipitates barium as barium carbonate. The unprecipitated barium may be determined quantitatively by titration with oxalic acid.

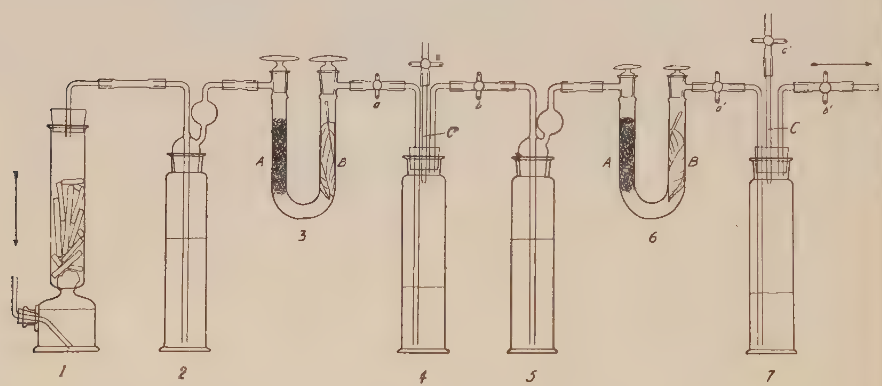


Fig. 19.—Apparatus for measuring respiration, modified from Sachs. Explanation appears in the text.

The apparatus consists, as shown in the accompanying illustration, of (1) a gas washing tower containing below a concentrated solution of caustic potash and above a number of sticks of caustic potash, (2) a gas washing bottle of caustic potash or barium hydrate, (3) a **U**-tube containing in arm A a wad of glass wool saturated with caustic potash. Arm B holds the leaves to be tested. (4) A gas washing bottle with an extra tube C for introducing liquids. (5) A washing bottle similar to 2. (6) A **U**-tube similar to 3. (7) A washing bottle similar to 4, and (8) a 20 litre aspirator to draw air through the whole series.

The bottles of this series are all connected by means of rubber tubing. Screw clamps for closing these tubes are pleaced at a, b, c, a′, b′, and c′. **U**-tubes 3 and 6 are alike, one being used for healthy leaves and one for diseased. Bottles 4 and 7 are alike. It is in these bottles that the actual testing is done. They will, therefore, be called test bottles, to distinguish them from the washing bottles, which are used solely to remove carbon

[1]Maige, A. Bul. Soc. Hist. Nat. Afrique Nord. 2:29. 1909.

dioxide from the air passing into the apparatus. The rubber stoppers of the test bottles are bored with three holes, one each for the inlet and outlet tubes and a third for a short glass tube C or C′ extending half an inch below the stopper and carrying a short piece of rubber tubing fitted with a screw clamp above. Through this tube the solutions necessary for the test are introduced, without opening the bottle.

The solutions necessary for the respiration tests are:

I.—A standard solution of oxalic acid so made that 1 c. c. will neutralize that amount of barium hydrate which can be precipitated by 1 c. c. of pure $CO_2$. The formula for such a solution is:

| | |
|---|---|
| Oxalic acid, (crystals) | 5.6325 g. |
| Water, (dist.) | 1,000.0000 c. c. |

II.—A solution of barium hydrate to receive the carbon dioxide respired by the leaves:

| | |
|---|---|
| Barium hydroxide | 6.00 g. |
| Barium chloride | .30 g. |
| Water, (dist.) | 1,000.00 c. c. |

This solution was made four litres at a time and kept in an aspirator bottle. To prevent carbon dioxide of the atmosphere from coming in contact with the solution this aspirator bottle was fitted with a U-tube containing pumice stone saturated with potassium hydroxide. The aspirator bottle was connected directly with a side arm filling burette which was also protected by a U-tube containing pumice stone and caustic potash. Even with these precautions the strength of the barium solution varied somewhat with age, and it was necessary to make frequent check titrations against the standard oxalic acid solution in order to obtain accurate results. The strength of the barium solution was not corrected when check tests showed variation, but the correction was made in the value of 30 c. c. of the barium solution in terms of oxalic acid on the daily record sheet. Check titrations were always made in the test bottles which were previously drawn full of air free from $CO_2$.

The manipulation of the apparatus is as follows: The whole series is connected up as shown in the figure with bottles 4 and 7, each containing 50 c. c. of distilled water and a few drops of aqueous phenolphthalein and with screw clamps, a, b, a′ and b′ open and c and c′ closed. The aspirator is then started by opening screw clamp d on the aspirator syphon, and so regulating it that about 15 to 20 bubbles of air per minute pass through the bottles. This is continued for two hours or more. The test bottles, 4 and 7, are thus filled with air which is free from carbon dioxide. Clamps a, b, a′ and b′ are then closed and the test bottles with their rubber connections disconnected from the rest of the apparatus. The test bottles are then taken to a burette and 30 c. c. of a stock solution of barium hydrate introduced

into each through tubes c and c′. In order to avoid spilling the reagents the burettes are connected directly with c or c′ by means of the rubber tubing. While introducing the reagents it is necessary to open clamps b or b′, slightly, to let the air out.

All the clamps are then quickly closed and the test bottles re-connected with the rest of the apparatus.

The apple leaves to be tested for respiration are then weighed out carefully and placed in the U-tubes, the diseased ones in arm B of tube 3 and the healthy in arm B′ of tube 6, or vice versa. Clamps a, b, a′ and b′ are then opened and the aspirator started as before. The apparatus is kept continually in the dark and is allowed to run for usually 12 hours. The syphon on aspirator 8 is so regulated that approximately 20 litres of air are drawn through the apparatus in 12 hours. The air entering the apparatus is deprived of its $CO_2$ by passing through the caustic potash in 1, 2 and arm A of 3. It then passes over the leaves in arm B. Some of the oxygen is there taken up and $CO_2$ is given off. This is drawn through test bottle 4 and precipitates some of the barium hydroxide as barium carbonate. The air passing out of 4 is further washed in 5 which also serves to remove $CO_2$ from any air admitted while the test bottles are disconnected. Passing over the leaves in B′, oxygen is again exchanged for $CO_2$, which is caught in test bottle 7. A minor question of the accuracy of the instrument might arise here. Some of the oxygen absorbed from the air in passing over the leaves in 3 is precipitated as $CO_2$, when it reaches the barium solution in 4. This being the case a slightly smaller amount of oxygen passes over the leaves in 6 than passes over those in 3. To avoid any errors from this source or from any other involving the relative position of the leaves in the apparatus the leaves were changed about occasionally. For several days diseased leaves were run in tube 3 and healthy ones in tube 6. Then for a like period the healthy leaves were run in tube 3 and the diseased in tube 6. The changes made no apparent differences in the results.

At the expiration of the 12-hour run, the screw clamps are all closed, the test bottles disconnected as before and taken to a burette of standard oxalic acid solution, against which their contents are titrated by admitting the acid through c and c′. The phenolphthalein introduced with the distilled water acts as indicator.

The value in terms of standard oxalic acid solution of the 30 c. c. of barium originally admitted to the test bottles is known by previous check titrations. The value of the unprecipitated barium in terms of oxalic acid is found by the above method. The difference between these two is the value of the barium in terms of oxalic acid which has been precipitated by the $CO_2$ respired by the leaves. Since 1 c. c. of the oxalic acid is equal to

1 c. c. of carbon dioxide, this difference may be read directly as c. c. of $CO_2$ respired, and it is so recorded on each of the daily record sheets.

A sample daily record sheet is presented herewith.

*Respiration Experiment No. 7.*

| Date—July 25, 1914. | Healthy | Diseased |
|---|---|---|
| Kind of leaves used: York Imperial | | |
| 30 c. c. Ba $(OH)_2$ in terms of oxalic acid | 12.5 c. c. | 12.5 c. c. |
| Unprecipitated Ba $(OH)_2$ in terms of oxalic acid | 5.9 c. c. | 5.5 c. c. |
| c. c. of $CO_2$ respired | 6.6 c. c. | 7.0 c. c. |
| Experiment began 7 P. M. | | |
| Experiment ended 7 A. M. | | |
| Duration, twelve hours | | |
| Weight of leaves | 2 g. | 2 g. |
| c. c. of $CO_2$ respired per g. of leaf per hour | .2750 | .2916 |

The results of the season's work are taken from the daily record sheets and presented in tabular form below.

TABLE XXVIII.—*Respiration of Diseased and Healthy York Imperial Apple Leaves.*

| Date, 1914 | Duration of experiment in hours | c. c. of $CO_2$ respired | | c. c. of $CO_2$ respired per gm. of leaf per hour | | Ratio of $CO_2$ respired by diseased leaves. Healthy=100 |
|---|---|---|---|---|---|---|
| | | Diseased | Healthy | Diseased | Healthy | |
| PART I | | | | | | |
| July 18 | 12 | 7.4 | 6.4 | .3083 | .2666 | 115.64 |
| 20 | 12 | 7.7 | 6.8 | .3208 | 2833 | 112.81 |
| 21 | 12 | 7.4 | 6.2 | .3083 | .2583 | 119.35 |
| 22 | 12 | 7.0 | 5.5 | .2916 | .2291 | 127.28 |
| 23 | 12 | 8.8 | 6.1 | .3666 | .2541 | 144.27 |
| 24 | 12 | 6.5 | 5.1 | .2708 | .2125 | 127.43 |
| 25 | 12 | 7.0 | 6.6 | .2916 | .2750 | 106.03 |
| 27 | 12 | 3.7 | 1.6 | .1541 | .0666 | 231.38 |
| 30 | 12 | 4.5 | 3.0 | .1875 | .1250 | 150.00 |
| 31 | 12 | 5.3 | 4.7 | .2208 | .1958 | 112.76 |
| Average | | | | .2720 | .2166 | 125.57 |

PART II

| | | | | | | |
|---|---|---|---|---|---|---|
| Aug. | | | | | | |
| 1 | 12 | 4.8 | 2.6 | .2000 | .1083 | 184.67 |
| 3 | 12 | 5.1 | 3.4 | .2125 | .1416 | 150.07 |
| 4 | 12 | 6.6 | 4.9 | .2750 | .2041 | 134.73 |
| 5 | 12 | 3.9 | 3.2 | .1625 | .1333 | 121.90 |
| 6 | 12 | 3.8 | 2.5 | .1583 | .1041 | 152.06 |
| * 7 | 12 | 4.2 | 3.6 | .3500 | .3000 | 116.66 |
| 8 | 12 | 4.6 | 3.7 | .3833 | .3083 | 124.32 |
| 11 | 12 | 4.1 | 3.2 | .3416 | .2666 | 128.13 |
| 12 | 12 | 5.2 | 4.4 | .4333 | .3666 | 118.19 |
| 13 | 12 | 4.7 | 3.8 | .3916 | .3166 | 123.68 |
| 14 | 12 | 3.0 | 3.3 | .2500 | .2750 | 90.90 |
| 15 | 12 | 6.3 | 5.0 | .5250 | .4166 | 126.02 |
| 16 | 12 | 2.6 | 2.2 | .2166 | .1833 | 118.16 |
| 19 | 12 | 3.8 | 1.8 | .3166 | .1500 | 211.06 |
| 20 | 12 | 6.5 | 4.1 | .5000 | .3153 | 158.57 |
| 22 | 12 | 5.5 | 5.1 | .4583 | .4250 | 107.83 |
| 23 | 12 | 7.8 | 7.2 | .6500 | .6000 | 108.33 |
| 24 | 12 | 5.7 | 4.2 | .4750 | .3500 | 135.71 |
| 26 | 12 | 3.5 | 2.6 | .2916 | .2166 | 134.62 |
| 27 | 12 | 3.8 | 2.7 | .3166 | .2250 | 140.71 |
| 28 | 13.5 | 2.7 | 2.8 | .2000 | .2074 | 96.43 |
| 31 | 6 | 2.0 | 1.3 | .3333 | .2166 | 153.87 |
| 31 | 12 | 2.5 | 2.2 | .2083 | .1833 | 113.63 |
| Average | | | | .3325 | .2614 | 127.19 |

PART III

| | | | | | | |
|---|---|---|---|---|---|---|
| Sept. | | | | | | |
| 1 | 7 | 2.5 | 2.3 | .3571 | .3285 | 108.70 |
| 1 | 12 | 2.2 | 1.5 | .1833 | .1250 | 146.64 |
| 3 | 12 | 3.4 | 3.2 | .2833 | .2666 | 106.26 |
| 4 | 6 | 1.1 | 2.1 | .1833 | .3500 | 52.37 |
| 5 | 12 | 2.8 | 2.3 | .2333 | .1916 | 121.76 |
| 7 | 12 | 1.0 | 0.7 | .0833 | .0583 | 142.88 |
| 8 | 12 | 3.4 | 3.5 | .2833 | .2916 | 97.15 |
| 9 | 12 | 1.4 | 1.4 | .1166 | .1166 | 100.00 |
| 10 | 12 | 2.0 | 1.3 | .1666 | .1083 | 153.83 |
| 14 | 17 | 1.8 | 2.3 | .1352 | .1058 | 127.78 |
| 17 | 12 | 1.1 | 2.3 | .0916 | .1058 | 86.57 |
| 18 | 12 | 3.4 | 3.1 | .2833 | .2583 | 109.67 |
| 19 | 10 | 2.9 | 2.1 | .2900 | .2100 | 138.09 |
| 21 | 12 | 2.3 | 2.7 | .1058 | 2250 | 42.53 |
| 23 | 12 | 2.1 | 2.7 | .1750 | .2250 | 77.77 |
| 24 | 12 | 3.5 | 4.0 | .2916 | .3333 | 87.48 |
| 25 | 12 | 3.7 | 2.7 | .3083 | .2250 | 137.02 |
| 28 | 24 | 4.8 | 5.7 | .2000 | .2375 | 84.21 |
| Oct. | | | | | | |
| 3 | 12 | 2.2 | 3.0 | .1833 | .2500 | 73.32 |
| Average | | | | .2081 | .2111 | 98.57 |
| Season's average | | | | .2708 | .2297 | 117.89 |

*Beginning Aug. 7 one gram of leaf was used in each apparatus instead of two grams as before that date.

The data presented in the foregoing table substantiate the results obtained with the Ganong apparatus, i. e., apple leaves diseased with cedar rust respire more rapidly and exhaust more quickly their food material available for respiration than do normal leaves. In about 15 hours diseased leaves usually cease to produce carbon dioxide entirely, while the healthy leaves may continue to do so for 48 hours or more. If both diseased and healthy leaves are allowed to function until respiration ceases entirely in both, the healthy leaves will produce a much larger total amount of carbon dioxide than the diseased leaves. An examination of the data obtained by both sets of apparatus during the latter part of the season, i. e., from September 1 on, will show some inconsistencies in the results. It will also be noticed that the amount of carbon dioxide given off by both diseased and healthy leaves decreases gradually as the season advances. The leaves, as they approach the end of their usefulness, work with less and less vigor until they finally turn yellow and fall off. The inconsistencies in the results during September are partly attributable to this fact. A part of the inconsistency may also be attributed to the fact that all the badly diseased leaves had fallen by September 1. After that it was necessary to use leaves bearing only 4 or 5 lesions or less. In a 12-hour test, for example (see data for September 21-24), the diseased leaves may exhaust their supply of starch in six hours and cease to respire. At the end of nine hours the healthy leaves may respire as much carbon dioxide as the diseased ones did in six, and in twelve hours may have forged ahead.

Nicolas[1] has obtained results in concordance with the above. A study of young and old leaves in about twenty species of plants showed a greater respiratory energy and a higher respiratory quotient in the young leaves. Nicolas attributes this difference to greater ease of penetration of gases to the young leaves.

Just how the parasite brings about an increase in respiration is not clear. Whether the increased respiration is confined to the cells of the rust lesion itself or is communicated to all the cells of the leaf is another question. There are three possible explanations which seem to cover the case. (1) A histological study of the lesions has shown that the palisade cells and those of the hypertrophied leaf parenchyma are almost if not quite devoid of both starch grains and chlorophyll. Consequently they must draw their food material from other normal adjoining cells by translocation. The fungus then in its growth and production of aecidiospores is continually drawing on this translocated food material. This brings about a constant streaming of food material from normal to hypertrophied cells, and from these in turn through the intercellular mycelium and aecidium into the spores which are being continually shed. The kinetic energy necessary to

[1]Nicolas, G. Bul. Soc. Hist. Nat. Afrique Nord. 7:109. 1910.

carry on this process must be generated by respiration, since no other source of energy is available.

(2) The parasite may develop toxins which diffuse outward and affect the respiration of the host tissue.

(3) The fungus may cause an increase of respiration by preventing the closing of the stomata. Histological studies and the results of transpiration tests support the idea that the stomata of diseased leaves are functionless, remaining partially open all the time, at least in the epidermis of the rust lesions themselves. It is possible that a paralysis of stomata is communicated to all parts of a diseased leaf by means of diffusible toxins or otherwise. If this be true the respiration of diseased leaves must necessarily be much greater in darkness than is the case with healthy leaves.

At any rate the diseased leaves are much less economical than the healthy ones.

The increased respiration of leaves diseased with cedar rust gives an insight into the cause of the poor growth and unthrifty condition of the affected trees. It is frequently assumed that respiration is in general a measure of growth intensity. This assumption will hold good for healthy organs, but in the case of disease the abnormally high respiration may be compared to a fever which wastes away the tissues of the victim.

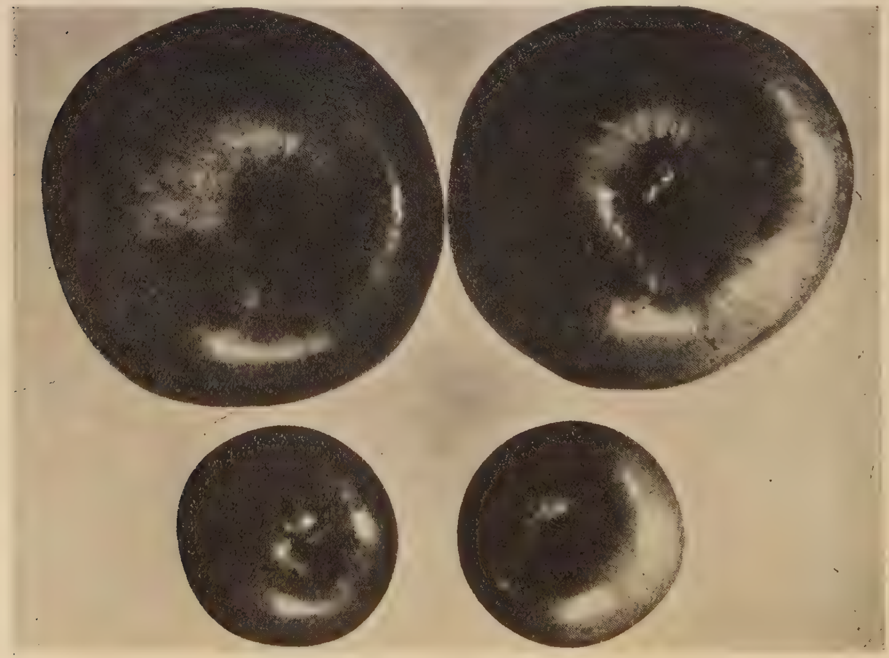

Fig. 20.—The results of spraying York Imperial with copper-lime-sulphur. The two lower apples came from a sprayed tree; the two upper came from an unsprayed tree in the next row.

## X.—SPRAYING EXPERIMENTS FOR THE CONTROL OF CEDAR RUST INFECTION ON THE APPLE FOLIAGE.

Spray treatments have been quite generally successful for controlling the foliage diseased caused by various sorts of fungi, and spraying has been practiced to a greater or less extent for the control of the cedar rust. At the outset of our work plans were made for a careful study of the value of various solutions for the control of this disease. The orchardists in the rust infested districts have practiced spraying for the common insect and fungous enemies of the apple for many years, but without marked success in controlling the cedar rust, although the opinion prevailed that spraying had more or less efficiency in controlling that disease.

### 1. Historical.

Reference to published literature shows that many of the earlier experiments for the control of this rust were not successful. Pammel[1] reported that he sprayed several wild crab apple trees for the control of this disease. Three applications were made; Bordeaux mixture being used for the first two and ammoniacal copper carbonate for the third. He found, however, little benefit from this spraying.[2]

Galloway[3] states with reference to an experiment with Bordeaux mixture on apples that the foliage remained fairly healthy, yet the benefit was not sufficient return for the labor expended.

Jones[4] described an experiment made in 1899 in which the apple trees received two applications of ammoniacal copper carbonate. He reported that there was no marked difference between the percentage of healthy leaves on sprayed and unsprayed trees, although the number of infections per leaf was somewhat reduced.

In a subsequent report[5] the same investigator states that a certain amount of protection to apple foliage was obtained by three applications of ammoniacal copper carbonate, but that it was not satisfactory when cedars stood near the apple trees.

Hein[6] reported results from three years' work, which showed no marked benefits from spraying. "During the present season cedars were carefully watched. After each gelatinous state of the cedar apples the apple trees under experiment were thoroughly sprayed. This spraying was done as soon as the rain, which caused the swelling of the cedar apples, ceased.

[1]Pammel, L. H. Iowa Agr. Exp. Sta. Bul. 84. 1905.
[2]Pammel, L. H. Iowa Agr. Exp. Sta. Bul. 13. 1891.
[3]Galloway, B. T. U. S. D. A. Rep't., p. 413. 1890.
[4]Jones, L. R. Vermont Agr. Exp. Sta. Fourth Report. p. 139. 1890.
[5]Jones, L. R. Vermont Agr. Exp. Sta. Fifth Ann. Rep't. p. 133. 1891.
[6]Hein, W. H. Insect Pest and Plant Disease Bureau of Nebraska. Cir. 1, 1908.

The most we can say in favor of the spraying is that the amount of rust may have been very slightly reduced."

Stone[1] reported spraying experiments which gave negative or indifferent results, but did not state the kind of spray material applied, nor the number of applications made.

Within the past few years more favorable results from spraying have been reported. This may be due to one or more of several conditions, e. g., better spray material, more successful machinery for spraying, better knowledge of the fungus, and hence more timely application of the fungicide.

Bartholomew[2] reports favorable results from spraying apple trees in the vicinity of Baraboo, Wisconsin. Three applications of Bordeaux mixture were given the foliage of Wealthy apple trees. When the first application was made there were very few tendrils extruded from the cedar galls; the second was made a week later when about half the gall was covered with the gelatinous tendrils; and the third, eight days later than the second when the gall was completely covered with the gelatinous tendrils. Three orchards were sprayed, one in close proximity to the cedar trees, one a quarter of a mile away, and the other a mile away. In all cases there were distinct benefits from spraying, the greatest benefit being apparent on the trees nearest the cedars. There was also a marked diminution in the number of infections per leaf.

Giddings and Neal[3] in a preliminary report also state that they found it possible to control the disease to some extent by only one application. Their experiments were made in the eastern part of West Virginia. Trees of the York Imperial and Ben Davis varieties were sprayed with Bordeaux mixture, lime-sulphur, and Atomic Sulphur. Applications were made on different dates from April 22d to May 29th. Considerable improvement, both in quality and quantity of the fruit, was found on certain of the trees. Their results showed that the applications made at certain dates were much more efficient in controlling the disease than others, and concluded that timeliness of application is of great importance in spraying for the control of this disease.

In another publication Giddings[4] minimized the value of spraying and emphasized that of destroying the cedar trees.

Stewart[5] had reported the outcome of spraying tests made by Sirrine on Long Island. Trees sprayed twice with Bordeaux mixture—June 1st and about two weeks later—showed much rust. In a subsequent season the spraying was done earlier and showed better results. The first application

[1] Stone, R. E. Alabama Agr. Exp. Sta. Cir. 2, 1908.
[2] Bartholomew, E. T. Phytopathology. 2:253. 1912.
[3] Giddings, N. J., and Neal, D. C. Phytopathology. 2:258. 1912.
[4] Giddings, N. J. Proc. W. Va. Hort. Soc. p. 20. 1913.
[5] Stewart, F. C. N. Y. Agr. Exp. Sta. Bul. 328. 1910.

was made on April 30th, as the blossoms were commencing to open; the second on May 21st. As a result, the sprayed trees showed only one-tenth as much rust as the unsprayed.

There are also numerous publications in which spraying is recommended as a control measure, but no experimental data are given in support of the recommendation.[1]

The only experiments in spraying the cedar tree appear to be those of Heald[2] who sprayed four blocks of small cedars with 6-6-48 Bordeaux mixture to which three pounds of soap were added. The results showed that spraying at intervals from the time that mature aecidiospores appeared until September first prevented infection and greatly reduced the number of "cedar apples." He further states, "The practical application of spraying cedars for the prevention of 'cedar apples' must largely depend upon local conditions. The reduction of the number of galls is not sufficient to be of much value in preventing the infections of adjacent apple trees, but if the life of valuable cedars is being threatened by the abundance of the fungus, spraying should reduce the ravages of the fungus sufficiently to prevent any material injury to the cedars."

Chase[3] sprayed Shockley apple trees with Atomic Sulphur and with lime-sulphur, but found neither substance of great value in controlling the cedar rust. Some benefit was derived from the spraying, but the results were not satisfactory enough to recommend the practice, and the destruction of neighboring cedar trees was advised.

Experiments upon the control of cedar rust and other foliage diseases of the apple were reported by Waite[4] in 1910, which showed some beneficial results from the use of spray materials. Three applications of spray materials were made on the Ben Davis, Yellow Newtown, *(Albemarle Pippin)* and York Imperial varieties. The spray materials used were,

Bordeaux mixture, (3-3-50).
Iron Bordeaux mixture, (3-3-50 plus two pounds of iron sulphate).
Bordeaux mixture plus gypsum, (3-3-50 plus three pounds of gypsum).
Neutral Bordeaux mixture.
Self-boiled lime-sulphur, (10-10-50).
Copper sulphide mixture, No. 1, (self-boiled lime-sulphur, 10-10-50, plus two pounds of copper sulphate).
Copper sulphide mixture, No. 2, (Bordeaux mixture, 3-3-50, plus one gallon of commercial lime-sulphur solution).
Iron sulphide mixture, (self-boiled lime-sulphur, 10-10-50, plus three pounds of iron sulphate).
Arsenate of lead, (2-50).

The author states, (p. 9), "All the fungicides protected the trees almost completely from fungous diseases. * * * It therefore became a

[1]Smith, J. B., and Halsted, B. D. N. J. Agr. Exp. Sta. Bul. 86. 1892.
Chester, F. C. Delaware Agr. Exp. Sta. 8th Rep't. 1896.
Butz, G. C. Pa. Agr. Exp. Sta. Bul. 43. 1898.
Smith, R. I., and Stevens, F. L. N. C. Agr. Exp. Sta. Bul. 206. 1910.
[2]Heald, F. D. Nebraska Agr. Exp. Sta. 22d Ann. Rep't. 1909.
[3]Chase, W. W. Georgia State Bd. Ent. Bul. 38. 1913.
[4]Waite, M. B. Bureau of Plant Industry. U. S. D. A. Circ. 58. 1910.

question, as was intended from the start, of determining the merits of the different mixtures largely through their effect in producing spray injury."

This experiment station published[1] a bulletin in 1912 in which brief reference was made to the utility of Bordeaux mixture for controlling cedar rust. Two years later another publication[2] was issued giving the results of more extended experiments. The data discussed in the bulletin mentioned will be treated below in a more exhaustive manner than it was possible to do in a bulletin designed to be more or less non-technical.

## 2. Experimental.

During the seasons 1910, 1911, 1912, and 1913, this department has conducted further spraying experiments upon the control of cedar rust in the northwestern part of the State. Orchard trees were kindly placed at our disposal by several parties near Harrisonburg, Middletown and Strasburg, Virginia. We wish to acknowledge our thanks, especially, to Messrs. Larrick and Larrick, Edgar MacDonald, A. Forney, and J. H. Pifer.

We selected in each orchard a block of trees of different varieties which were near enough to cedar trees to be exposed to considerable chance of infection. In fact, all the trees were known to have been more or less infected and injured by the disease in previous years.

a. Spray materials employed.—These experiments included the trial of a variety of spray materials, the efficiency of which is herewith discussed. The effect of spraying at various periods of development is treated in a subsequent section.

*Lime-sulphur.*—This material has recently found much favor as a summer spray for various fungous diseases of the apple. Its utility has been discussed in two of the former publications of this experiment station.[3] This material has been applied in the dilute form as a summer spray, and the results have been closely studied for four years. One and a half gallons of the concentrated commercial solution, testing 32 degrees on the Beaumé spindle, was diluted to 50 gallons for use. Arsenate of lead was added to the solution for the early applications. This is the strength ordinarily used in orchard spraying in this State. In the majority of cases this material gave protection against cedar rust, if applied early enough. It adheres well to the apple leaves and causes a minimum of spray injury.

*Iron-lime-sulphur.*—This is a spray solution like the preceding with the addition of three pounds of iron sulphate (copperas) to each 50 gallons. The addition of this compound increased the adhesive properties of the spray material. This material, when applied to the trees, gave the foliage

[1]Reed, H. S.; Cooley, J. S., and Rogers, J. T. Va. Agr. Exp. Sta. Bul. 195. 1912.
[2]Reed, H. S.; Cooley, J. S., and Crabill, C. H. Va. Agr. Exp. Sta. Bul. 203. 1914.
[3]Va. Agr. Exp. Sta. Bul. 188. 1910, Bul. 195. 1912.

a dark brown aspect. From a distance it appeared as though the leaves had been scorched, but upon close examination no injury could be found. It was possible to find traces of it on the leaves in the autumn at the time of picking the fruit. The efficiency of this spray was quite similar to that of lime-sulphur alone, although in rainy weather it was somewhat greater, due to the fact that it stuck better to the leaves.

*Copper-lime-sulphur.*—This spray material was made by adding copper sulphur (bluestone), to the ordinary dilute lime-sulphur at the rate of two pounds per 50 gallons.[1] This made a muddy brown spray, due to the formation of copper sulphide. On most trees, when copper-lime-sulphur was used, it was possible to find leaves whose margins were much darker green than the normal leaf, but no evidence of scorching by this material was found.

This material gave the greatest protection against cedar rust of any of those tried. The superiority of this spray was especially evident in 1911 in the MacDonald orchard. The sprayed trees showed almost no cedar rust infection, averaging only three spots per leaf; while the unsprayed trees had an average of 111 spots per leaf. In 1912 this material did not give quite as good results, due, probably to the fact that hard rains washed it off too soon. This difficulty was overcome in 1913 by the addition of one-half gallon of " black strap " molasses to each 50 gallons of spray material.

*Atomic Sulphur.*—This is a trade preparation which was claimed to have especial value in controlling cedar rust. It is sold in the form of a paste, which appears to be very finely ground sulphur, and when required for use is mixed with water at the rate of 10 pounds to 50 gallons of the latter. This material appears to have about the same value for the control of cedar rust as ordinary lime-sulphur, but it did not adhere to the foliage as long. After two years' experiment, we did not find it enough better for this purpose than ordinary lime-sulphur solution to warrant the higher price charged for it.

*Bordeaux mixture.*—This is the regular 3-4-50 Bordeaux, made of 3 pounds copper sulphate (bluestone), four pounds unslaked (stone) lime, and 50 gallons water, and applied as soon as made.

During the past few years nearly all of the apple growers in this State have discarded Bordeaux mixture for the dilute lime-sulphur solution, because of the freedom from spray injury when the latter is used. However, Bordeaux mixture is still the standard spray for control of the bitter rot of apples. The fruit of such varieties as Ben Davis, Arkansas, *(Mammoth Black Twig)*, Jonathan, Baldwin, Gano, usually suffer quite extensive russeting of the skin when sprayed during their early stages with Bordeaux mixture. On the foliage various types of injury, such as spotting, curl, or

[1]The bluestone was dissolved in three gallons of water, which was then added to the dilute lime-sulphur and stirred in.

scald may be produced. These types of spray injury and their causes have been discussed in former publications[1] and need not be repeated here. Injury from spotting the leaves was quite common on the Bordeaux plat of our experiments in 1913.

Bordeaux mixture has shown itself to be generally effective in controlling cedar rust. Wherever copper compounds are present in the spray mixture they appear to give noticeable protection against the cedar rust. This may be in part due to the fact that sprays like Bordeaux and copper-lime-sulphur have good adhesive powers, but we do not believe that all the benefit can be acounted for in that way.

In our experiments in 1910 and again in 1912, we found from 5 to 10 percent less rust on trees sprayed with Bordeaux mixture than on those sprayed with lime-sulphur. Yet on account of the danger from spray injury, we do not recommend Bordeaux mixture for the control of cedar rust.

*Iron Bordeaux mixture.*—This is the regular 3-4-50 Bordeaux mixture to which 3 pounds of iron sulphate (copperas) were added. This gave the mixture a brown color which was very noticeable on the foliage of the trees. The addition of the iron sulphate increased the adhesive property of the mixture, but did not alter the question of spray injury. This formula for Iron Bordeaux seems to have been first used by Selby of the Ohio Agricultural Experiment Station, and has also been tried by Waite.[1] This material has practically the same efficiency in controlling cedar rust as the regular Bordeaux, although at times we have found its better adhering properties have made it slightly more efficient.

In 1912, we found that the use of Bordeaux and Iron Bordeaux caused some spray injury on the apple leaves. This injury was manifested only on those leaves which were young at the time of spraying, and later showed infection with the rust fungus. It seems probable that rust infection had taken place just before the sprays were applied, and that the dissolved copper entered the leaves through the opening which the fungus hyphae had made. These leaves showed very slight spray injury soon after they were sprayed, and about June 28 they began to turn yellow. On July 3 one to three leaves had fallen from many twigs.

*Mercuric Bichloride.*—This material was tried in the experiments one year with fairly good success. It is a substance having great germicidal powers and consequently much used as a disinfectant in surgery. We sprayed York apple trees with a solution containing one part of the mercuric bichloride in a thousand of water without injury to the foliage, but with good results in diminishing the amount of cedar rust. It must be stated,

[1]Reed, H. S. Va. Hort. Soc. Ann. Rep't. 1911.
Reed, H. S.; Cooley, J. S., and Rogers, J. T. Va. Agr. Exp. Sta. Bul. 195, 1912.
[1]Waite, M. B. Bureau Plant Industry. U. S. Dept. Agr. Cir. 58. 1910.

however, that the season was marked by scanty rainfall, and that the amount of infection was small, and also there was little chance for this material to be washed from the foliage. The trees on which the spray material was applied were without a crop of fruit, consequently no observations upon spray injury on the fruit could be made. It is not assumed that the use of mercuric bichloride is practical in a commercial way.

b. RESULTS OF SPRAYING EXPERIMENTS.—The following tables give a presentation of the results of the spraying experiments made in 1911 and 1912 near Middletown, including all the spray materials used. In the following tables each figure is the average of several records.

The column headed " total " gives the average of the total number of infections on the leaves of that season's growth. The column headed " relative" represents the relative amount of infection based upon a figure of 100 for the unsprayed twigs.

Table XXIX.—*Results of Spraying Experiments. York Trees. MacDonald Orchard. 1911.*

Leaf Numbers.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Total | Relative |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Check—Unsprayed | .5 | .1 | 0 | 0 | 0 | 0 | .0 | .1 | 1.0 | 9.4 | 20.8 | 23.3 | 16.4 | 11.5 | 4 | 1.0 | 88.1 | 100 |
| I and II—Lime Sulphur | .1 | 0 | 0 | 0 | 0 | .1 | .1 | 3.7 | 5 | 14 | 23.7 | 35.7 | 24 | 3.6 | .5 | 0 | 111.4 | 126 |
| I, II and III—Lime Sulphur | .05 | 0 | 0 | 0 | 0 | .05 | .0 | .05 | .5 | 6.4 | 14 | 12.5 | 9.1 | 3 | .05 | 0 | 45.7 | 52 |
| I, II and III—Atomic Sulphur | .1 | .1 | 0 | 0 | 0 | .1 | .3 | 1 | 4.4 | 5 | 9.4 | 13.5 | 9.6 | 11.2 | 5.1 | .6 | 60.4 | 69 |
| I, II and III—Bordeaux | 0 | 0 | 0 | 0 | 0 | .1 | .05 | 2.5 | 2.5 | 10.3 | 4.6 | 1.5 | .8 | 1.7 | 0 | 0 | 24.05 | 27 |
| I, II and III—Iron Bordeaux | 0 | 0 | 0 | 0 | 0 | 0 | .0 | 1.1 | 5.3 | 10.0 | 5.9 | 5.5 | .1 | 0 | 0 | 0 | 27.9 | 32 |
| I, II and III—Copper Lime Sulphur | .1 | .1 | 0 | 0 | 0 | 0 | .0 | 0 | .15 | .1 | 0 | .5 | .1 | .45 | 0 | .1 | 1.6 | 2 |



Table XXX.—*Results of Spraying Experiments. York Imperial Trees. Larrick & Larrick Orchard. 1911.*

Leaf Numbers.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Total | Relative |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Check—Unsprayed | .2 | .2 | .1 | 0 | 0 | 1.2 | 2.0 | 3.6 | 5.6 | 8.7 | 14.0 | 6.5 | 6.2 | 5.6 | 3.3 | 0 | 57.2 | 100 |
| I—Lime Sulphur | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.8 | 12.3 | 26.1 | 27.1 | 7.5 | 1.7 | 0 | 0 | 0 | 76.5 | 134 |
| I, II and III—Iron Lime Sulphur | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.2 | 6.4 | 15.7 | 19.2 | 13.0 | 10.6 | 4.3 | .5 | 0 | 70.9 | 124 |
| I, II and III—Lime Sulphur | .1 | 0 | 0 | 0 | 0 | 0 | .0 | 1.6 | 4.2 | 11.5 | 14.6 | 17.8 | 3.5 | .9 | 0 | 0 | 54.2 | 95 |
| I, II and III—Atomic Sulphur | 0 | 0 | 0 | 0 | 0 | 0 | .0 | .3 | 3.8 | 17 | 14.4 | 9.3 | 1.8 | .1 | 0 | 0 | 44.7 | 78 |

Application I was made on April 26th, when the cluster buds on the York Imperial trees were well unfolded, but lateral buds were still closed. About three days later the blossoms began to unfold, and a week later the trees were in full bloom. Application II was made on May 16th, about a week after the bloom fell. At that time the trees had a good covering of leaves, many of which had reached full size. Application III was made on June 1st, and was really the most important application for that season, because the heaviest infection began on the day on which the spray material was applied.

An inspection of the vertical columns in the table shows that the first two leaves on each twig examined showed a few rust infections, but that the following leaves were quite free from infection until the eighth or ninth leaf is reached. As in the case of the unsprayed tree the bulk of infection occurred on six leaves which were unfolded just previous to the heavy infection beginning on June 1st.

An inspection of the horizontal rows of figures shows the relative efficiency of spray materials in preventing cedar rust infections in this orchard.

Applications I and II of Lime-sulphur ($1\frac{1}{2}$ to 50) had apparently no efficiency in controlling the disease, because they were applied too far in advance of the single heavy infection of that year. These trees appeared to have more rust infection than the unsprayed tree. The next figures showing the effect of applications I, II, and III, show how important application III was, since the relative amount of infection was thereby reduced from 126 to 52, thus substantiating what was said above.

Atomic Sulphur was found to be somewhat less efficient than ordinary lime-sulphur applied under similar conditions. Bordeaux mixture and the Iron-Bordeaux mixture were both quite similar in efficiency and appeared in this orchard to control cedar rust better than lime-sulphur.

Copper-lime-sulphur was by all odds the best fungicide employed in this experiment. It gave practically clean foliage and produced no spray injury either to foliage or fruit.

The results of another set of spraying experiments in the Larrick and Larrick orchard in 1911 are shown in Table XXX, in which the same method of computing was employed.

Application I, was made on April 27th; II, on May 17th; and III, on June 3d. The stage of development of the apple trees was practically the same as described for the trees in the MacDonald orchard.

All the spray mixtures here used were sulphur compounds, and it will be noted that none were very efficient in controlling the disease. One important reason for the poor effect may be due to the fact that application III (here also the critical application) was not made until June 3d, thus allowing two or three days for infection to occur. It will be noted that leaves

10 to 13 inclusive received quite heavy infection. In these experiments, Atomic sulphur shows up somewhat better than in those presented in the foregoing table, indeed, it was the only material which showed much efficiency.

The poor showing made by application I, of lime sulphur, is undoubtedly due to its being applied over a month in advance of infection.

The season of 1912 was marked by a more abundant rainfall at Middletown than that of 1911, and the increase during May was considerable. The Weather Bureau of the United States Department of Agriculture furnished us with records of the rainfall at Harpers Ferry, W. Va., which showed that the rainfall for May, 1911, was .58 inch, while that for May, 1912, was 3.29 inches. In 1912, a station was established at Winchester, Va., twelve miles from Middletown, and the record of that station shows rainfall of 5.94 inches during May, 1912.

The amount of infection of apple foliage was therefore greater and thus tested more severely the efficiency of spray treatments.

Records kept at Middletown by ourselves show that in May, 1911, rain fell on one day; in May, 1912, on eight days; and in May, 1913, on six days.

In 1912, plots of York Imperial trees were sprayed again in the Larrick and Larrick and in the MacDonald orchards.

The spray materials used were practically the same as those employed in the previous year and the concentrations were similar. The spraying was always done with a large pump driven by a gasoline engine. The results of the sprays were judged by the relative amount of infection on the foliage as shown by the figures presented in Table XXXI.

TABLE XXXI.—*Effect of Spray Materials in Preventing Foliage Infection. York Trees. Larrick & Larrick Orchard. 1912.*

| | Leaf Numbers. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Total | Relative |
| Check—Unsprayed | 1.6 | 5 | 11 | 22 | 33.6 | 33.6 | 18 | 13 | 8 | 3 | .4 | .5 | .3 | 0 | .1 | 0 | 150.1 | 100 |
| I, II and III—Copper Lime Sulphur | .08 | 1.7 | 8 | 22 | 23 | 26 | 25 | 19.5 | 2.8 | .2 | 0 | 0 | 0 | 0 | 0 | 0 | 128.28 | 85 |
| I, II and III—Bordeaux | 1 | 12 | 6 | 13 | 14.5 | 20 | 9 | 2.3 | .3 | .1 | 0 | 0 | 0 | 0 | 0 | 0 | 78.2 | 52 |
| I, II and III—Iron Bordeaux | .9 | 2.3 | 5.9 | 7.7 | 13.5 | 12.8 | 7.3 | 3.1 | 1.0 | 0 | .2 | .2 | .5 | 0 | 0 | 0 | 55.4 | 37 |

TABLE XXXII.—*Average Amount of Infection on Foliage of York Trees. MacDonald Orchard. 1912.*

| | Leaf Numbers. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Total | Relative |
| Check—Unsprayed | .06 | .3 | 4.4 | 1.5 | 22 | 36 | 44 | 27 | 10.3 | .8 | .6 | .5 | 0 | 0 | 0 | 0 | 147.46 | 100 |
| I and II—Copper Lime Sulphur | 0 | 0 | 0 | 1.4 | 7 | 14.4 | 22.4 | 12.8 | 4.4 | .4 | 0 | 0 | 0 | 0 | 0 | 0 | 62.8 | 43 |
| I and II—Lime Sulphur | 0 | .2 | 4.5 | 6.5 | 15 | 11 | 9.5 | 6.6 | .8 | .8 | 1.3 | .5 | 0 | 0 | 0 | 0 | 56.7 | 38 |
| I and II—Bordeaux | .2 | .2 | 1.6 | 7.2 | 8 | 5.4 | 2 | .55 | .4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25.55 | 17 |

Application I, was made on April 20th, which was the second fair day after a six-day rainy period. The cedar apples were in a highly gelatinous condition and the teleutosori contained large number of sporidia. The apple foliage buds were just unfolding. This application was made two days before any blossoms opened, and four days before the trees were in "full bloom."

Application II was made May 10th, at which time the petals had fallen from the apple trees and the young shoots had three or four leaves of five cm. length. In all the plots a few cedar rust spots could be found on the "cluster leaves," i. e., those which unfolded first, due to infection which had taken place just before the first spraying.

Application III was made on May 27th, just prior to a two-day rain, on which date it was evident that many leaves had been infected between the first and second spraying during a cloudy, rainy period from May 7th to 12th. There were at this time eight to ten leaves on the new growth, and two to four leaves on each, which had not been previously coated with spray material.

Considerable spray injury was manifest at this time on foliage which had been sprayed previously with Bordeaux and Iron Bordeaux. The leaves in question showed "spotting" like that previously described by one of the writers.[1] The injury was confined to leaves which were young and tender at the time the spraying was done.

An inspection of the table shows that all of the spray materials employed decreased the amount of cedar rust infection, but that the relative efficiency of the different materials was somewhat different from that of the preceding year, since Bordeaux and Iron Bordeaux were both more efficient than Copper-lime-sulphur. It is believed that these results are primarily due to the difference in adhesiveness of the spray materials employed, and the reason that the copper-lime-sulphur was less efficient is because the copper sulphides it contains do not have as great a covering power as the copper and iron hydroxides of the other spray materials. There were hard rains of one to two days duration immediately after applications I and II had been made, which undoubtedly removed much of the copper-lime-sulphur. This idea is also supported by the serial increase in average number of infections per leaf. The figures indicate that the later leaves received a decreased amount of protection, especially after the effects of the first application were lost.

Later examination on July 1st, showed that the trees sprayed with copper-lime-sulphur had a healthy green color, in spite of the numerous cedar rust spots, but those sprayed with Bordeaux and Iron Bordeaux showed many yellow leaves and considerable defoliation. The injury seemed

[1] Reed, H. S. Va. Hort. Soc. Rep't. 1911.

to have been mainly caused by the second spraying, and was due, not to bad spray material, but to the period of rainy weather which followed.

Experiments made in the MacDonald orchard in 1912, gave quite similar results to those just related. Bordeaux mixture was more efficient than either lime-sulphur or copper-lime-sulphur. The figures giving the amount of infection on foliage in July are given herewith in Table XXXII.

On these plots only two applications of spray materials were made. Application I was made April 21st, and II was made May 6th. The development of the trees was similar to that noted above for the plots in the Larrick and Larrick orchard.

The statements made above concerning the relation between efficiency and adhesiveness appear to hold for these plots also. The figures showing the amount of infection on leaves show that those which received application I, i. e., the first leaves to unfold, were well protected thereby, because the average number of rust infections per leaf is considerably less than on the trees sprayed with other compounds or not sprayed. The reason for this is that little rain fell between Applications I and II, but immediately after II (May 6th) there was a week of cloudy, rainy weather. Coincident with these conditions there is a material increase in the number of infections on the fifth, sixth, and seventh leaves. On the trees sprayed with Bordeaux mixture, the number of infections fell off quite rapidly from the seventh leaf on. This is what might be expected since it is known that the efficiency of Bordeaux mixture increases with the continued action of meteorological factors.

Spraying experiments were continued in 1913 using blocks of York Imperial trees in the same orchards. In the Larrick and Larrick orchard, plots of trees received the following sprays: Bordeaux mixture, Iron Bordeaux, Sulfocide, Lime-sulphur (summer strength), Copper-lime-sulphur, and Copper-lime-sulphur plus molasses. This last material was made by adding one-half a gallon of " black strap," a cheap grade of molasses, to each 50 gallons of Copper-lime-sulphur prepared as described previously. The purpose of adding the molasses was to increase the adhesiveness of the spray material.

The first application of spray materials was made on April 29th, after the trees had blossomed, but while still a few petals remained on the trees. The second application was made May 12th, when there were six to seven leaves on the new growth. On that date a few spermogonia had made their appearance on the unsprayed control trees. No rain fell between the first and second applications, but rain fell on seven days between the second (May 12th) and third (June 1st) applications. Observations made on May 30th showed, however, that most of the spray materials had adhered well. The humid, rainy weather had, however, brought on more or less

spray injury, especially where copper containing mixtures had been applied to the trees.

In this season the records were carefully made, but the system of counting was not followed as in the previous two years. Observations made on July 2d showed that there was least cedar rust on trees sprayed with lime-sulphur, but the copper-lime-sulphur was nearly as good. The addition of molasses to the copper-lime-sulphur made it adhere well. The spray material was still plainly to be seen as a steel-blue coating on the foliage. Bordeaux mixture and Iron Bordeaux, although apparently less efficient than lime-sulphur, controlled the cedar rust very well. The trial of Sulfocide was not satisfactory, since on account of lack of material only one application was made.

Further notes on these trees taken on August 29th, state that the foliage sprayed with Bordeaux mixture or Iron Bordeaux had suffered more spray injury than that receiving other applications. At that time many leaves had fallen as a result of the injury. The trees sprayed with lime-sulphur were exempt from spray injury and showed very little cedar rust infection on the foliage. The unsprayed control trees had lost considerable foliage as a result of the cedar rust infection.

In the same season a block of 25 trees in the MacDonald orchard was sprayed. The trees used were in a corner of the orchard, and there were cedar trees about one-fourth mile to the southeast. Plots contained one Grimes, one Ben Davis, two York Imperial, and one Arkansas tree, had each received the following treatment: Lime-sulphur, copper-lime-sulphur, Bordeaux mixture, Iron Bordeaux, unsprayed controls. Applications were made April 30th, May 13th, and June 3d. A rainy period of four days followed the application of May 13th, and caused considerable spray injury on the foliage receiving mixtures containing copper in any form; however, the application of fungicides at this particular time undoubtedly prevented a great deal of infection.

Notes made on July 18th, show that there was practically no rust infection on any of the varieties which had been sprayed as outlined above, but on trees 50 yards away, which received only one application of lime-sulphur, there was considerable rust. The only trees in the unsprayed plot to show much infection were the York Imperials.

c. Summary.—The results of our work indicate that spraying is generally effective in controlling the cedar rust provided that the applications are made at the right time. If young apple leaves can be coated with spray mixture soon after they unfold, and kept coated until the first week in June, spraying will be effective in preventing infection. In the case of the York Imperial it is unnecessary to spray until the trees have blossomed, although the spraying may well be done while a few petals still cling to the

trees. In the case of varieties like Jonathan and Arkansas Black, which put forth their foliage in advance of the blossoms, it may be necessary to spray before the blossoms appear.

Subsequent applications of spray materials should be made often enough to protect new leaves as they appear.

The greatest efficiency in preventing infection is obtained when the spray material is applied, just prior to rainfall, or as soon thereafter as possible. A delay of one day may allow so much infection as to render the spray ineffective.

The spray materials found to have greatest efficiency were Bordeaux mixture, Iron Bordeaux, Lime-sulphur, and Copper-lime-sulphur, the preparation of each of which has been described above. Other things being equal, the more adhesive materials are the more efficient.

Spray injury was observed on trees sprayed with materials containing copper, if rainy weather of several days duration followed the applications.

Since the time in which effective spraying may be done is so limited, it is generally impractical to spray all the trees in a large orchard at the proper time, and it is more economical to destroy the neighboring cedar trees. A discussion of this question is given in another section.

## XI.—THE PROBLEMS OF CONTROL OF THE DISEASE.

The prime motive in most of our studies has been to find the most practical means of controlling this disease. The orchard industry, which is large in this State, has suffered severely in the past from cedar rust. Certain districts in which the orchards are largely composed of York Imperial and other susceptible varieties of apples, were the worst sufferers. Orchards in the vicinity of cedar thickets have usually suffered more severely than those situated at some distance. (Figs. 21 and 22.) The injury is more marked if the orchard is on the leeward side of the cedar trees where the sporidia may be continually borne to them by prevailing winds, but at the season of spore production the direction of the winds is quite variable, and all orchards of susceptible varieties are more or less completely infected, regardless of position. In 1912 alone, the damage to the crop was estimated to be in the neighborhood of five hundred thousand dollars.

The actual injury to the orchards was, however, much greater than this, because the premature defoliation shortened the growing period of the trees and impaired greatly the vitality of the fruit buds for the following year. If the infection is severe, the foliage begins to fall from the trees early in August, and often the trees are almost destitute of leaves by the first of September. The precise effect upon transpiration, photosynthesis, and respiration is noted in another section. In any locality where abundant infection has occurred year after year, the trees are in a generally feeble

Fig. 21.—The effect of cedar rust from neighboring cedars. This York Imperial tree has lost most of its foliage.

Fig. 22.—Tree in same orchard as that shown in Fig. 21, but located far enough from cedar trees to escape severe infection. Note the healthy condition of the foliage.

condition, and are often attacked by numerous other diseases due to both insects and fungi. In some cases the trees have perished before arriving at the bearing age. Sometimes a tree 17 to 20 years old is not much larger than a normal tree at five years.

Under these circumstances the necessity of practical means of control of the disease has been a matter of prime importance both to the plant pathologist and to the apple producer. The attempted solution of the problem has been principally along the following lines: (a) Spraying the cedar trees, (b) spraying apple trees, (c) destruction of cedar trees, (d) removing the galls from cedar trees, (e) planting resistant varieties of apples.

Spraying the cedar trees with a combined Bordeaux and soap solution in the late summer and fall was tried by Heald,[1] as noted previously. The treatment was fairly effective in preventing infection in the season in which the trials were made. On a large scale the plan would, however, be very difficult to carry out, because much of the cedar growth in Virginia is on rough, steep land where spray machinery could not be used. The practice of spraying the cedar trees in the spring has also been suggested. This would require that they be sprayed immediately after each rain which caused a gelatinization of the teleutospore tendrils, and the operation would be prohibitive on account of the cost of materials and labor required, although it might be applied to a few ornamental trees, in which case the motive would be to save the cedar trees rather than to protect the apples.

Spraying the apple trees is effective in certain cases, especially if the applications can be combined with those required for other fungous and insect diseases. The foregoing section on spraying has presented the results of work which show the effectiveness of spraying with various materials, provided always that they are applied at the proper time. In a small orchard this is not a serious consideration, but the case is different in a large one. The time in which effective spraying may be done is not long enough to allow one to cover a large area effectively without an undue outlay for machinery and labor. We have shown that a delay of one or two days may determine whether a spray treatment shall be effective or otherwise. There are many situations in which effective spraying may be done and where this will probably always be the most profitable way of controlling the disease. Cases where this is effective are the following: Orchards far enough from cedar trees to escape severe infection, orchards containing varieties which are only moderately susceptible to the cedar rust, small orchards, and other special cases.

Eradication of the cedar trees is obviously an efficient although somewhat radical remedy for the disease. Most of the writers upon the problem

[1]Heald, F. D. Nebraska Agr. Exp. Sta. Ann. Rep't. 22:105-113. 1909.

of cedar rust control have advised this as the most practical means of control.[1]

The experience of nearly all students of the problem in the eastern and southeastern States has shown that eradication of the cedar trees is the most economical remedy and the only one giving permanent relief from the disease. This is particularly true in the orchard district in the Shenandoah Valley where the red cedar grows abundantly on all land which is undisturbed by the plow for a few years, and where the loss to the districts from cedar rust has amounted to a far greater sum than the value of the neighboring cedar trees.

In 1913, we made germination tests of the teleutospores from galls on cedar trees, one, two, three, and four months after they had been cut. The results showed that many galls retained their ability to produce viable teleutospores two months after the trees had been cut. It is therefore advisable to burn any trees cut later than March 1st.

The distance through which it is necessary to remove cedar trees in order to insure the safety of the orchards, naturally varies according to local conditions. Since the sporidia causing infection are carried only by air currents, it is obvious that the existence of barriers which will deflect the currents will lessen the amount of infection. Again, the direction of prevailing winds makes cedar trees on the windward side of an orchard a more prolific source of infection than trees situated at a similar distance on the leeward side of an orchard. If all cedar trees can be removed from a territory having a radius of half a mile from the orchard, we have found that the serious epidemics of cedar rust will be avoided, but it is much better to destroy all cedars within a mile of the orchard, and in some cases where the topography is such that winds have an unbroken sweep, two miles is the least distance which will make the orchards safe.

A small amount of infection may be borne from almost any distance, but if the above limits are observed, the orchard will escape serious injury.

The practice of destroying the cedar trees has been conspicuously successful because it has led to the destruction of trees growing close to the orchard along boundary fences, in little thickets on waste places, and along

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[1]Among others the following may be noted:
Smith, J. B. and Halsted, B. D. N. J. Agr. Exp. Sta. Bul. 86. 1892.
Alwood, W. B. Va. Agr. Exp. Sta. Bul. 17. 1892.
Kinney, L. F. R. I. Agr. Exp. Sta. Rep't. 1894.
Chester, F. D. Del. Agr. Exp. Sta. 8th Rep't. 1896.
Butz, G. C. Pa. Agr. Exp Sta. Bul. 43. 1898.
Jones, L. R. and Giddings, N. J. Vermont Agr. Exp. Sta. 19th Ann. Rep't. p. 232. 1905-06.
Pammel, L. H. Ia. Agr. Exp. Sta. Bul. 84. 1905.
Hein, W. H. Insect Pest and Plant Dis. Bur. Nebraska. Circ. 1. 1908.
Smith, R. I. and Stevens, F. L. N. C. Agr. Exp. Sta. Bul. 206. 1910.
Price, H. L. Va. Agr. Exp. Sta. Circ. 7. 1909.
Lloyd, F. E. and Ridway, C. S. Ala. Dep't Agr. Serial No. 39. 1911.
Giddings, N. J. Proc. W. Va.. State Hort. Soc. 1913.
Chase, W. W. Ga. State Bd. Ento. Bul. 38. 1913.
Reed, H. S.; Cooley, J. S., and Crabill, C. H. Va. Agr. Exp. Sta. Bul. 203. 1914.

roadsides. (Fig. 23.) Most of these trees were mere bushes having little, if any, commercial value.[1]

The fungus, *Gymnosporangium juniperi-virginianae*, occurs on the native red cedar, *Juniperus virginiana L.*, but has been found also on *Juniperus virginiana, var. Scottei, Juniperus virginiana var. glauca*, and *Juniperus sabina, var. fastigiata* in a nursery in this State.

**Fig. 23.—The red cedar trees are often abundant along roadsides in Virginia. The illustration shows a typical view.**

In making plantings for ornamental purposes, it would be advisable, therefore, to avoid using these species and varieties.

The only dangerous parts of a cedar tree, so far as the propagation of the rust is concerned, are the galls, or "cedar apples." It is, therefore, possible to control the disease by removing all the galls some time in advance of the season at which teleutospores will be produced. This has been done quite successfully in a number of cases. The method is only to be commended, however, in the case of ornamental cedars on private premises, in parks, or cemeteries where it is impossible or impractical to destroy the trees. The work must be done systematically and thoroughly every year.

The galls removed from the cedar trees should be burned.

The method would not be practical if the number of cedar trees were great, or the trees themselves were large, since it is impossible to find all the galls on large trees with dense foliage.

[1]A number of prominent orchardists in Virginia having been convinced of the necessity and desirability of eradicating cedar trees from the vicinity of apple orchards, succeeded in getting legislation on the subject at the 1914 session of the General Assembly of Virginia. The text of the law is given in Circular No 9 of the Virginia State Crop Pest Commission, Blacksburg, Va.

Resistant or immune varieties of apples are known and are more or less extensively cultivated in this State. In some cases this is a desirable means of obviating losses from the cedar rust disease. We have not attempted to make an extensive list of susceptible and immune varieties in this State, but the following list classifies the more important commercial varieties.

*Susceptible.*

York Imperial.
Northern Spy.
Rome.
Jonathan.
Bonum.
Smith's Cider.

*Moderately Susceptible.*

Ben Davis.

*Resistant.*

Northwestern Greeting.
Winesap.
Stayman Winesap.
Arkansas.
(Mammoth Black Twig.)
Grimes.
Yellow Newtown.
(Albemarle Pippin.)

In New Jersey the difference in susceptibility of certain varieties of apples was noted by Ellis[1] in 1883. He noted that the Baldwin and Fallawater foliage was heavily infected, but Summer Sweet, Yorkshire Russet, and English Russet were only slightly infected.

Chester has given[2] an extensive classification of varieties with respect to their susceptibility to infection. In the main our observations agree with those of Chester. We find though that Albemarle Pippin is very slightly affected. Since this is a synonym for the name Newtown Pippin and Chester reports the latter to be free from rust, it would appear that the so-called Albemarle Pippin should be transferred to the same class as Newtown Pippin. We would also transfer Smith's Cider to the "Badly Affected" list.

The following lists are taken from Chester's paper:

*Lists of Varieties of Apples and Amount of Rust Found upon the Leaves.*

BADLY AFFECTED.

Albemarle Pippin.
Bonum.
Bostick Queen.
Burt.
Carolina Red June.
Carter's Blue.
Carthouse.
Clayton.
Doctor Walker.
Fallawater.
Four Belle Flower.
Golden Russet.
Grimes Golden.
Haywood.
Jackson.
Johnson.
Jones Cider.
Lanier.
Lankford.
Magnum Bonum.
Maryland Blush.
Minkler.
Missouri Pippin.
Nero.
Nickajack.
Red June.
Rhodes Orange.
Roman Stem.
Rome Beauty.
Shannon.
Scarlet Cranberry.
Shockley.
Simmons Red.
Smokehouse.
Stark.
Summer Pearmain.
Tallman Sweeting.
Two Large Red.
Western Romanite.
York Imperial.

[1]Ellis, J. B. Amer. Nat. 17:1281. 1883.
[2]Chester, F. D. Del. Agr. Exp. Sta. 8th Rep't. 1896.

SLIGHTLY AFFECTED.

| | | |
|---|---|---|
| Abram. | Drap d'Or. | Lily of Kent. |
| American Golden Russet. | Gano. | Maiden's Blush. |
| Akin. | June. | Red Cain. |
| Ben Davis. | King. | Rhode Island Greening. |
| Carolina Beauty. | Lanber. | Twenty Ounce. |
| Crawford. | Lankford Seedling. | Yates. |

FREE FROM RUST

| | | |
|---|---|---|
| Arch. | Haughley. | Red Astrachan. |
| Arkansas Black. | Hargrove. | Red Bietigheimer. |
| Black Twig. | Holland Pippin. | Smith's Cider. |
| Buncombe. | Kinnarid. | Springdale. |
| Cowgill. | Knowles Early. | Stayman's. |
| Early Colton. | Lily of Kent. | Strawberry. |
| Early Harvest. | Limber Twig. | Summer Queen. |
| Early Lippincott. | Mann. | Sweet Bough. |
| Fall Pippin. | McAfee. | Taunton. |
| Fall's Favorite. | Newtown Pippin. | Wagner. |
| Fonville. | Niack Pippin. | White Winter Pearmain. |
| Gloria Mundi. | Paragon. | Winesap. |
| Golden Pippin. | Peter. | Winter Brown. |
| Gravenstein. | Rawle's Jannet. | Winter Gricksome. |
| Hagloe. | | Yellow Transparent. |

Another classification of varieties, according to their rust susceptibility in Alabama, is that given by R. E. Stone.[1] Our classification differs from his, mainly in the position of the York Imperial. In Virginia this is one of the most susceptible of apples, while Stone places it under the head, "Slightly affected."

In conclusion, we would state that the problems of control of the cedar rust disease are not simple, and that it is necessary for each one to determine the best method of procedure in his own special case. Eradication of the cedars will give effective and permanent relief, spraying is successful under the conditions set forth above, and planting of resistant or immune varieties is much to be commended.

[1]Stone, R. E. Ala. Agr. Exp. Sta. Circ. 2. 1908.

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